# Optimizing nursery rearing, outplanting, sexual reproduction and innovative artificial materials for coral restoration in French Polynesia

Camille Leonard

PSL UNIVERSITÉ PARIS

CRIOBE UAR3278

École Pratique des Hautes Études | PSL | cnrs | UP VD

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# Perfectionnement des phases de pépinière, transplantation, reproduction sexuée et matériaux artificiels innovants à des fins de restauration corallienne en Polynésie française

## Optimizing nursery rearing, outplanting, sexual reproduction and innovative artificial materials for coral restoration in French Polynesia


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Composition du jury :

| | |
|---|---|
| Christine FERRIER-PAGES<br>Directrice de recherche, CSM | *Présidente* |
| Paola FURLA<br>Professeur, Université Côte d'Azur | *Rapporteur* |
| Sebastian FERSE<br>Directeur de recherche, Leibniz-ZMT | *Rapporteur* |
| Lucie PENIN<br>Maître de conférences, IRD | *Examinatrice* |
| Maggy NUGUES<br>Maître de conférences, EPHE | *Directrice de thèse* |
| Laetitia HEDOUIN<br>Directrice de recherche, CNRS | *Co-directrice de thèse* |

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PhD Thesis

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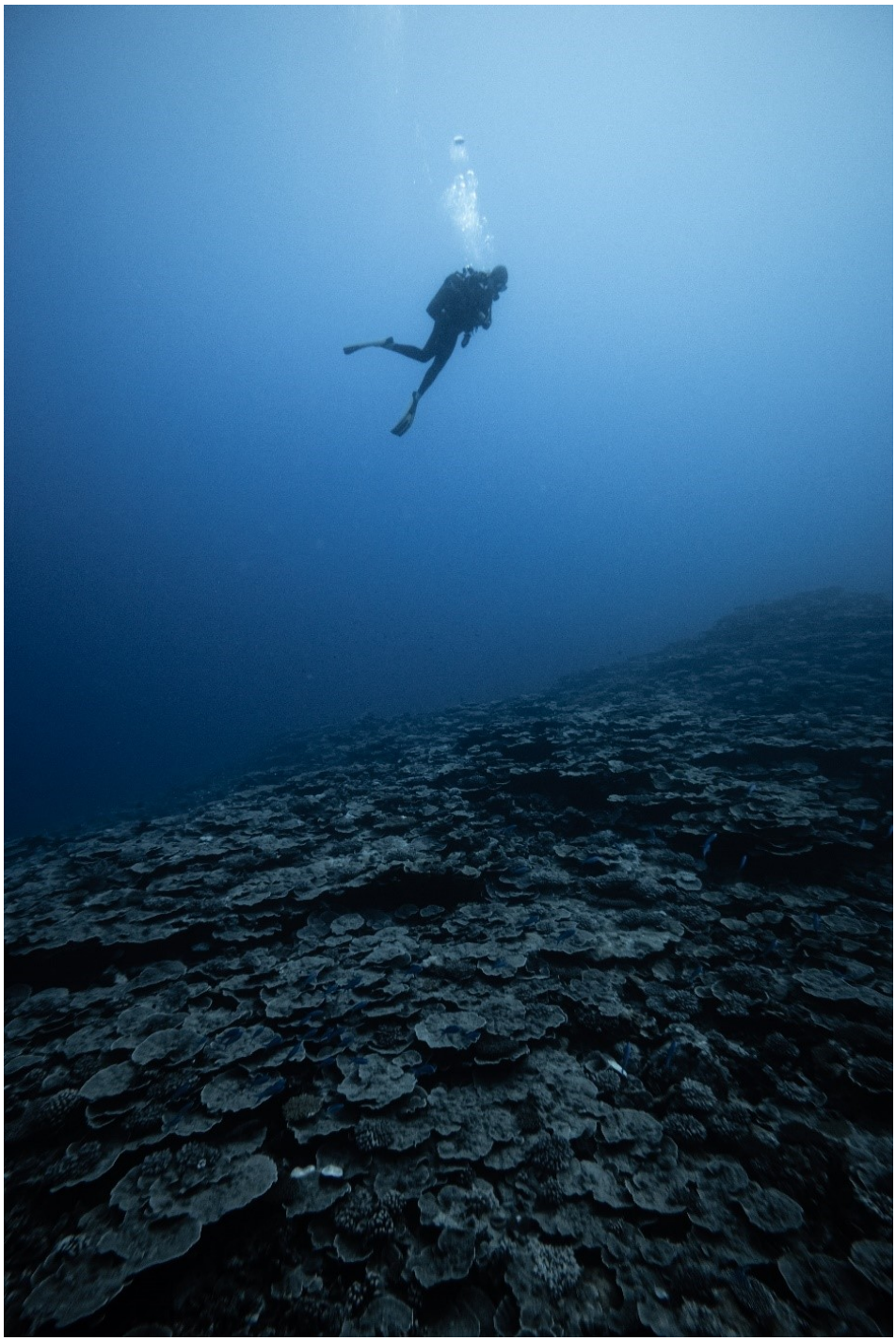
By
Camille Leonard

Center for Island Research and Environmental Observatory (CRIOBE)
École Pratique des Hautes Études (EPHE)
Paris Sciences et Lettres (PSL)



*“Do you see the way that we have gone too far*
*We need now more than ever before*
*To come together put our differences apart*
*Stop drifting off of our course*
*Do you see the way that we need our reefs just like trees along the shore*
*If it knows to help*
*Half of what we breathe in for ourselves*
*Is out of sight and on the ocean floor*
*Oh, how much there is left to learn”*

*Ziggy Alberts*

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# Table of contents

# List of publications and communications

## Publications

- **Leonard C**, Hédouin L, Lacorne M, Dalle J, Lapinski M, Blanc P, Nugues MM (2021) Performance of innovative materials as recruitment substrates for coral restoration, Restoration Ecology
- ***Leonard C**, Hédouin L, Stojakovic J, Davezies I, Dalle J, Lapinski M, Blanc P, Nugues MM (2025) Optimizing surface complexity to enhance coral recruitment on artificial materials, in preparation for Restoration Ecology*
- ***Leonard C,** Davezies I, Stojakovic J, Hédouin L Physiological acclimation to nursery habitat variation in South Pacific Staghorn corals, in preparation*
- ***Leonard C,** Dubé C, Stojakovic J, Derome N, Hédouin L (2025) Transplantation of nursery-reared Acropora cytherea alters growth, survival and symbiont assemblages independently of varying environmental gradients, in preparation*
- ***Leonard C**, Becker D, Conetta D, Stojakovic J, Lacube Y, Hédouin L (2025) Enhancing Acropora sp. coral settlement: Insights from wild and nursery-reared spawning patterns and larval seeding strategies, in preparation*
- ***Leonard C**, Hédouin L, Stojakovic J, Davezies I, Dalle J, Lapinski M, Blanc P, Nugues MM (2025) Coral settlement success on artificial materials is not synonymous with long-term recruit survival, in preparation*

## Communications on the PhD subject

- Talk at the ECRS 2024 Napoli, titled 'Optimizing nursery rearing, outplanting, sexual reproduction and innovative artificial materials for coral restoration in French Polynesia'
- Virtual participation at ICRS 2022 Bremen (abstract available in Book of Abstracts at https://www.icrs2022.de/scientific-program/conference-schedule, and video available at https://youtu.be/ldyzq_6OnME)
- Chapter called 'Artificial reefs to increase coral recruitment' in book 'Étonnants récifs' by Laetitia Hédouin, CNRS Editions
- Poster and oral presentations at the PhD meetings at the University of French Polynesia, 2021 and 2022
- Appearance in CNRS video https://youtu.be/EjkxJpQuc3k
- Interview https://www.fnr.lu/research-with-impact-fnr-highlight/spotlight-on-young-researchers-saving-the-coral-reefs/
- Science festival video interview https://www.youtube.com/watch?v=Hfqdgmnjo0M&t=85s

## Publications outside of the PhD subject

- ***Leonard C**, Fellous A, Boitel C, Davezies I, Bonpain C, Bischoff H, Hédouin L (2025) Disparities in spawning time between shallow and mesophotic Porites rus in South Pacific reefs, in preparation*

*Abstract:* Porites rus is a very common coral of the Pacific living in lagoons and forereefs (down to the mesophotic zone) and even in some cases being the dominant species in reef landscape. P. rus colonies living in lagoons and mesophotic reefs have contrasted morphologies, mainly due to changes in light availability. Lagoon colonies harbor a massive shape with short branches by contrast to mesophotic colonies forming wide

and thin plates, called “roses”, and forming when dominant the so-called “reef rose gardens”. Our objective was to better understand the reproductive biology of P. rus living in these contrasted environments, focusing on the spawning patterns and reproductive output. Our work showed that lagoon colonies spawned 5 days After the Full Moon (AFM) over 5 consecutive months (from November to March), one and a half hours after sunrise. The spawning of mesophotic colonies occurred 5 days AFM, but on a reduced period (one or two months) with a 3-hour delay. We hypothesize that this delay could be driven by the reduced amount of light, and the reduced period of spawning could be due to energy constraints at deeper sites. Size of eggs ranged from 137 µm to 189 µm, with no difference between lagoon and mesophotic environments. Fertilization rate within populations averaged 25.2 and 14.9% after 3 hours. Unfertilized eggs were negatively buoyant and stayed viable for approximately 3 hours. However, sperm remained viable for more than 5 hours after its release. This resulted in the capacity of inbreeding between branch and plates colonies, with lagoon sperm fertilizing mesophotic eggs with the same fertilization success rate as mesophotic sperm. This suggests that the geographically and phenotypically distinct populations remain capable of interbreeding. Elongated swimming planulae were observed 4 days after fertilization. These observations further our understanding of the reproductive patterns of understudied mesophotic coral populations.

- ***Leonard C**, Hédouin L (2025) First observation of Acropora granulosa spawning in French Polynesia, South Pacific, in preparation*

*Abstract:* The timing and spawning patterns of many coral species are yet to be elucidated, especially for less common or less accessible species, such as those dwelling in mesophotic ecosystems. We report here for the first time the spawning day, time and reproductive output of Acropora granulosa in French Polynesia. In Mo’orea, this species is only found below 20 m depth. Laboratory spawning was confirmed two days after the March new moon, 2h41 following the sunset. This corresponds to the warmest month in French Polynesia. Contrastingly, other congeneric species are known to spawn between September and November, at the start of the warm season. The delayed spawning in A. granulosa could be explained by its deeper habitat, linked to a lower light availability and lower maximum temperatures. More research is needed to better understand the diversity of spawning patterns in less represented species, which would prove useful for management actions and coral restoration using sexual reproduction.

## Communications outside of the PhD subject

- Teaching a course on coral taxonomy to EPHE master students (2020 and 2021)
- Opening a marine educational area with Hao elementary and middle school (19/6/21-26/6/21). Press coverage https://www.tahiti-infos.com/Les-enfants-de-Hao-receptifs-a-l-avenir-du-recif_a210196.html and https://la1ere.francetvinfo.fr/polynesie/une-aire-marine-educative-bientot-a-hao-1297364.html. Follow up from 26/4/22-30/06/22 on the field and teaching three new classes, with a progress report written containing recommendations on activities to pursue with the classes the following year.
- Science Fest 2020, 2021, 2022: Teaching classes entitled ‘Introduction to coral reefs’ and ‘Reproduction of corals’ in elementary, middle and high schools in Tahiti, and Rangiroa Middle School. Public workshops in Papeete and at CRIOBE.
- Interview in movie ‘Turning Tides’ by Rachel Moore and Emmett Sparling, available at https://www.youtube.com/watch?v=BDdVQHgVbV4&t=1s

## Training

- **Bioinformatics** (29/08/22-09/09/22) at the Ifremer in Vairao (Learn how to use a computing cluster, assembling and annotating genomes, comparative genomics, analysis of metabarcoding data

# List of figures

# List of tables

# List of abbreviations

AFM – after the full moon

Anova – Analysis of variance

AR – artificial reef

CCA – crustose coralline algae

COTS – crown-of-thorn sea star

EPB – eggs per bundle

ERA – encrusting non-coralline red algae, for instance *Peysonnellia* sp.

ES – egg size

FGP – fiberglass-epoxy polymer

GHG – greenhouse gas

GLMM – generalized linear mixed model

KW – Kruskal-Wallis test

NGO - non-governmental organization

PC – porous concrete

PLA – polylactic acid

p, pval – p-value

PVC – polyvinyl chloride

pW – pairwise Wilcoxon test

RCP – representative concentration pathways (Hoegh-Guldberg et al. 2018)

SCTLD – stony coral tissue loss disease

SEM – standard error of the mean

# Chapter 1

## Introduction

## 1. Importance of coral reefs

Tropical coral reefs are biogenic constructions forming lush marine ecosystems. They are found either as fringing reefs all along the coasts of over 100 countries, as barrier reefs further away from the coast, as atolls when the initial volcanic island has disappeared, or as continental or oceanic coral banks (Davis 1928). They are usually situated in warm tropical waters, but can extend to latitudes as high as 33° (Yamano et al. 2001). Coral reefs are per unit area the most diverse ecosystems on the planet. They represent less than 0.1% of the earth's surface, an area smaller than mainland France (Reaka-Kudla 1997; Knowlton et al. 2010). Zooxanthellate corals, the builders of the structure of reefs, are, however, represented by less than 1000 species worldwide (Cairns 1999). Instead, it is the abundance of organisms living in and around the corals that make up the incredible diversity of the ecosystems (Knowlton et al. 2010). They potentially harbor up to one-third of all marine species, many of them still undescribed (Knowlton et al. 2010). This incomparable species richness makes coral reefs extremely valuable to the inhabiting fauna and flora, as well as to humans through the ecosystem services provided. Ecosystem services are the contributions that ecosystems make to human quality of life. For example, coastal inhabitants have always relied on coral reefs for provisioning through fishing, for building materials and for cultural activities (Woodhead et al. 2019). More recently, the promising pharmacological properties of bioactive molecules found on coral reefs are also raising growing interests (Debitus & Guézennec 2014).

Most importantly, coral reefs are essential for coastal protection by effectively dissipating destructive wave energy (Ferrario et al. 2014). Without barrier reefs and their protected lagoons, shoreline housing and constructions would be impossible (Woodhead et al. 2019). Indonesia, the Philippines, Malaysia, Mexico and Cuba are predicted, thanks to their coral reefs, to save over USD 400 million annually on flood damage repair (Beck et al. 2018). Additionally, reefs significantly reduce the severity of tsunamis (Kunkel et al. 2006), which can have devastating effects on more exposed coastal regions. Over 200 million people living near reefs benefit from risk reduction through these reefs, and would suffer significant costs if reefs were to be degraded (Ferrario et al. 2014).

Coral reefs are also very attractive for the tourism sector in numerous countries. Their white beaches, peaceful lagoons, islets and unique wildlife attract some 70 million tourists annually, making global reef tourism worth USD 35.8 billion every year (Spalding et al. 2017). Overall, the value of all ecosystem services provided is estimated to reach an incredible sum of USD 2.7 trillion per year (Souter et al. 2021), which makes a single hectare of coral reef worth on average over 100’000 USD per year. It is thus critical to advocate for the protection of coral reefs if we want to continue benefitting from the numerous ecosystem services they provide.

## 2. Biology and ecology of reef-building corals

Coral reefs are formed by scleractinian corals depositing calcium carbonate skeletons, growing on top of each other to build complex and solid structures. They work in concert with

octocorals, hydrozoans and calcifying algae, which effectively cement and stabilize coral skeletons (Littler & Littler 1984). Scleractinian corals, also called stony corals or hard corals, belong to the Cnidaria phylum, and can be split into hermatypic corals, which usually are zooxanthellate and reef-building, and ahermatypic or non-reef-forming corals, which are mostly azooxanthellate. Although solitary non-attached species exist, most species of reef-building corals are colonial organisms, composed of individual polyps which multiply through asexual budding (Veron & Stafford-Smith 2000).

Reef-building corals owe their success and rapid growth to an obligate endosymbiosis with photosynthetic algae of the Symbiodiniaceae family also called zooxanthellae, allowing them to thrive in nutrient-poor waters (Muscatine & Cernichiari 1969). Together with the nutritional input of photosynthesis, corals also rely on some heterotrophy, by capturing and ingesting bacteria and zooplankton, for their intake in nitrogen and phosphorus (Houlbrèque & Ferrier-Pagès 2009). As reef-building corals survive thanks to their symbionts, their distribution is heavily dictated by depth and light availability (Kahng et al. 2010). To this day, the deepest photosynthetic coral has been found in Mangareva, French Polynesia, at 172 m depth (Rouzé et al. 2021). Symbionts are critical for a coral's growth and fitness, and their diversity can be linked to their host's thermal response. For example, clade D, now called *Durusdinium* sp., provides a higher thermal tolerance but a slower growth than clade C or *Cladocopium* sp. (Jones & Berkelmans 2010). Symbiont shuffling, that is the uptake of different symbiont communities, can occur during high temperature and high nutrient concentration events (Rouzé et al. 2019), or when moved to a drastically different environment such as a land-based nursery (Gantt et al. 2023).

The symbiosis with zooxanthellae can be especially fragile under stress. Heat waves for instance are responsible for widespread bleaching events in corals. A prolonged heat stress will cause Symbiodiniaceae to release reactive oxygen species, which are toxic to their hosts (Downs et al. 2002). After expulsing their symbionts, corals lose both their nutritional resource and their pigmentation, thus appear bleached (Glynn 1996). This has detrimental effects on their growth and reproduction, and prolonged bleaching ultimately leads to the colony's death (Glynn 1996). If the temperature stress is short, corals can retrieve their lost symbionts and recover (Coles & Brown 2003). Bleaching can also be a strategy for a rapid acclimation to new conditions, allowing corals to acquire more favorable partners (Baker 2001). Destructive mass bleaching events have been reported since the 1980's and are predicted to become more widespread and more frequent (Hughes et al. 2017b). In only 12 years between 1979 and 1990, 60 major bleaching events have been reported, in every coral reef region (Glynn 1993). Since then, three pan-tropical bleaching events have hit coral reefs with unprecedented severity, in 1998, 2002 and 2016 (Hughes et al. 2017b). Out of over 600 surveyed reefs, 55.3% experienced bleaching in 1998, 57.6% in 2002 and 91.1% in 2016 (Hughes et al. 2017b). Following such bleaching events, coral reproduction and recruitment can be drastically reduced (Ward et al. 2002; Johnston et al. 2020), and fertilization rates can significantly decrease (Omori et al. 2001).

## Coral reproduction

Corals have varied reproduction strategies. Colonial corals grow through asexual reproduction, by budding new polyps. The growth can range from only 1 cm/year in massive corals such as *Porites* sp. to 18 cm/year in branching *Acropora* sp. (Dullo 2005). In some branching corals, broken branches dispersed by storms or predatory fish become new colonies nearby (Wallace 1985), forming large fields of clonal corals locally. While asexual reproduction through fragmentation and even parthenogenesis is common in some coral species (Ayre & Miller 2004), sexual reproduction is crucial for maintaining genetic diversity and for long-distance dispersal (Harrison 2011).

Spawning patterns of reef-building corals have long been a puzzle to scientists. The discovery of a synchronous mass spawning event on the Great Barrier Reef in Australia (Harrison et al. 1984) encouraged an acceleration of the study of coral reproduction worldwide. Since then, spawning patterns have been described in around 50% of coral species (Harrison 2011; Baird et al. 2021), though many remain unknown. Also, timing and even reproductive strategy can vary within the same species between geographic locations (Harrison 2011), which makes comparing reproductive studies tricky. Corals have a bipartite life history; a benthic adult phase and pelagic larval phase, and reproduce through internal fertilization in the case of brooding corals, or external mass release of gametes for broadcast spawners (Harrison 2011). They can be hermaphroditic or gonochoric when colonies have separate sexes (Harrison 2011). Reproductive output, which can be measured in the number of eggs per surface area, egg size or egg nutritional reserves, is dependent on the coral's morphology, size (Álvarez-Noriega et al. 2016) and its reproductive strategy. While spawning studies are crucial for elucidating fundamental aspects of coral ecology, a precise understanding of the spawning timing of threatened corals is also critical for local coastal management (Baird et al. 2021).

After internal or external fertilization of coral gametes, the embryo turns into a larva called a planula. Some inherit photosynthetic symbionts directly from the mother colony, some take up symbionts from the water column, but planulae are also able to feed on organic matter through an oral pore (Fadlallah 1983; Hartmann et al. 2017; Rodd et al. 2022). Larvae can survive for a considerable amount of time in the ocean, up to 100 days for *Pocillopora* (Harii et al. 2002) and 209 days for *Acropora* (Graham et al. 2008), allowing dispersing to distant reefs. However, they rarely spend more than a couple of days swimming freely (Miller et al. 2020), because mortality is high, and they generally look for a suitable settling spot rapidly. Thus, the actual dispersal distance of corals could be much lower than expected (Shinzato et al. 2015; Zayasu et al. 2016; Palumbi et al. 2023). Some larvae are able to metamorphose before settling, and if settling conditions are not optimal, can even revert to a mobile stage three days after settling (Richmond 1985).

The settlement behavior of coral larvae onto solid substrate can be influenced by biotic and abiotic factors, which include hydrostatic pressure, open ocean and local currents, water soluble and insoluble chemicals, reef sound, temperature, photosynthetically active radiation,

ultraviolet radiation and sedimentation (Gleason & Hofmann 2011). For instance the shape, orientation, size and even color can influence the colonization of a substrate (Spieler et al. 2001; Mason et al. 2011), and these drivers can vary across different coral families (Gouezo et al. 2020). All this suggests that coral larvae have highly developed senses, being able to see light and colors (Babcock & Mundy 1996; Mason et al. 2011), sensing dissolved metabolites (Gleason et al. 2009) and even directing themselves towards reef sounds (Vermeij et al. 2010). The presence of adult coral colonies and crustose coralline algae (CCA) are known to facilitate coral recruitment (Vermeij 2005). In contrast, other epibenthic organisms, such as *Aplysina* sp. sponges, can inhibit coral recruitment (McCook et al. 2001; Brandt et al. 2019). Macroalgae can also increase the mortality rate of coral recruits through allelopathic effects (Bulleri et al. 2018; Beatty et al. 2018; Fong et al. 2019). After finding an adequate settling spot, the planula metamorphoses into a polyp with a mouth and tentacles, and starts secreting a carbonate calcium skeleton (Gleason & Hofmann 2011).

Recruit mortality can be as high as 55% on the first day post-settlement (Martinez & Abelson 2013). Post-settlement mortality rates are this high because recruits are vulnerable to external pressures, such as predation by herbivores (Ritson-Williams et al. 2009; Christiansen et al. 2009), especially parrotfishes (Penin et al. 2010) or urchins (O'Leary et al. 2013), and competition with sessile organisms (e.g. sponges, ascidians, bryozoans) (Mundy 2000). In addition, coral recruits can be smothered by turf algae trapping sediment particles (Birkeland et al. 1981). The presence of grazing fish can, however, increase coral recruitment by controlling algal density (Brock 1979; Evensen et al. 2021). Predation is usually the main factor influencing post-settlement community structure (Spieler et al. 2001). To escape predation, coral larvae choose to settle into sheltered and cryptic microhabitats (Mundy 2000). Therefore, the availability of shelter is critical for the success of recruitment. Crevices on natural surfaces can be shaped by dead coral skeletons or parrot fish bite marks for instance (McDevitt-Irwin et al. 2023). Coral recruit density and diversity are usually correlated with the irregularity of the substrate (Carleton & Sammarco 1987). Nonetheless, there is still a knowledge gap on how coral recruitment is affected long-term by a combination of settlement substrate, surface complexity and benthic communities. Once they reach a certain size-escape threshold, usually above 30 mm, coral recruits become less subject to predation, and their survival chance consequently increases (Raymundo & Maypa 2004; Doropoulos et al. 2012).

Size and age typically determine the onset of maturity in corals (Hall & Hughes 1996), but other parameters can influence the growth and the onset of sexual maturation, leading to difficulty in predicting coral maturity (Randall et al. 2020). For example, while sexually propagated *Acropora* need to reach three years and a 12 cm diameter before being mature (Baria et al. 2012; dela Cruz & Harrison 2017), asexually propagated fragments of mature corals can spawn at a much smaller size (Rapuano et al. 2023). In slow-growing massive corals, maturity can be reached at around five years and 4 cm diameter, but the maximum fecundity was only reached after 15 years and 16 cm diameter (Babcock 1991). Together with the low recruit survivorship, this makes their generation time over 33 years long (Babcock 1991), possibly explaining the

low rate of speciation observed in corals (Potts 1984). Maturity can be strongly impacted by disturbances, such as predation (Rotjan & Lewis 2008), accidental fragmentation (Zakai et al. 2000) or heat stress (Szmant & Gassman 1990; Levitan et al. 2014). The favorable conditions of a balanced environment are thus necessary for the healthy reproduction and development of corals.

## 3. Threats on coral reefs

Despite their crucial importance, corals are currently the most endangered group of animals on earth; now over one-third of all corals are at risk of extinction, because of local threats and in particular climate change (Carpenter et al. 2008). Global coral cover has declined by more than half since the 1950s, with an irreversible loss of over 63% of the associated biodiversity (Eddy et al. 2021).

### Natural threats

The first widespread concern about the fate of coral reefs arose during an outbreak of *Acanthaster* sp., the crown of thorn sea star (COTS), in Guam in 1967 (Chesher 1969). This sea star, which comprises at least four species, is a voracious predator of corals that appears in periodic population outbreaks (Pratchett et al. 2017). Overfishing of *Charonia tritonis* has been suggested as an aggravating factor of COTS outbreaks. However, this carnivorous mollusk also feeds on other prey, and often leaves half on the COTS uneaten (Chesher 1969). This results in the survival and regeneration of the sea star, thus an abundance of *Charonia tritonis* might not prevent these natural outbreaks (Chesher 1969). In Mo'orea, French Polynesia, a massive COTS outbreak killed the majority of corals between 2006 and 2009, reducing the overall coral cover from 40% to less than 5% (Kayal et al. 2012).

Other natural causes of significant coral mortality include tropical storms, through associated strong swells and intense rainfalls. In the Northern Great Barrier Reef, cyclone Nathan caused a 90% coral cover drop in 2014, especially through the loss of more sensitive branching morphologies (Baird et al. 2018). Mortality in tagged *Acropora* and *Goniastrea* reached 97%, and sub-lethal effects were observed in a drop in fecundity and egg carbon content (Baird et al. 2018). Extreme floodwaters following cyclone Joy in 1990 caused 85% mortality in shallow corals on Keppel Island on the Great Barrier Reef, especially in Acroporidae and Pocilloporidae (Van Woesik et al. 1995). In French Polynesia, cyclone Oli in 2010 destroyed the skeletons of corals eaten by COTS, completely flattening the reef complexity thus removing the habitats of various reef organisms (Kayal et al. 2012).

## Anthropogenic threats at a local scale

In addition to natural threats, tropical coral reefs are being degraded worldwide due to combinations of various anthropogenic stressors (Hughes et al. 2017a). Historically, land reclamation, meaning the process of creating new land from the sea, urban expansion, coastal constructions, pollution and overfishing have all had dramatic consequences on coral reefs (Nakano 1990; Edinger et al. 1998; Hughes et al. 2013). Thus, ecological impacts need to be considered before major constructions in order to avoid irreversible damage (Maragos 1993). Boat anchoring, although of lesser impact, also contributes to coral reef degradation and loss of habitat complexity (Rogers & Garrison 2001; Flynn & Forrester 2019). In addition, dynamite or blast fishing, which is often illegal but still common in East Africa, the Philippines or Indonesia, can be especially destructive for coral reefs, and necessitate long recovery times (Fox & Caldwell 2006; Hampton-Smith et al. 2021).

Next to physical degradations, pollution through sewage and agricultural runoff enhances nutrient concentration in coastal waters, which is beneficial for macroalgae growth. These are direct competitors of corals and reduce coral recruitment rates (McCook et al. 2001). Nutrient rich runoff can also contribute to the formation of harmful algal blooms, also known as red tides (Walsh et al. 2006). These toxic blooms cause high coral mortality, for instance in Pocilloporidae in the Gulf of Oman (Foster et al. 2011). Additionally, nutrient pollution can significantly aggravate the outcome of coral diseases (Bruno et al. 2003). Consequently, nutrient pollution is negatively correlated to coral species richness and coral cover (Duprey et al. 2016). However, nutrient enrichment is not the primary factor necessary for a phase shift towards algal dominance on a coral reef, as this can occur after a natural disturbance and loss of coral cover, combined with a loss of herbivorous fish controlling algal overgrowth (Done 1992; McManus & Polsenberg 2004).

Overfishing constitutes a significant cause of coral decline. A targeted fishing of grazing parrotfish for instance causes a herbivore depletion in the ecosystem, which is usually followed by a phase shift of the reef in favor of macroalgae (Done 1992; Hughes et al. 2007) or other benthic animals (Norström et al. 2009). A dominance of macroalgae can harm corals through competition and reduce coral recruitment (McCook et al. 2001; Beatty et al. 2018). After a disturbance, such as a COTS invasion or a bleaching event, the abundance of remaining herbivores will be directly correlated to the recovery and resilience of corals (Hughes et al. 2007). Thus, a strict control of fishing practices targeting herbivorous fish is critical for the prevalence of coral reefs (Adam et al. 2015).

## Anthropogenic threats at a global scale

On a much wider scope than local pollution, emergent coral diseases strongly shape coral populations in some parts of the world. Originally present only around Miami, Florida (Walton et al. 2018), stony coral tissue loss disease (SCTLD) is currently spreading throughout all Caribbean reefs (Brandt et al. 2021). SCTLD can cause similar reductions in coral cover as

bleaching events (Brandt et al. 2021). The pathogen is likely bacterial, but remains to be identified (Neely et al. 2020). The disease travels with ocean currents and shows a very rapid spread through reefs of around 155 m/day (Truc et al. 2023). Boat ballast waters and biofilms, as well as dive tourism, are suggested to contribute to the rapid spread of the disease, and isolated islands remain the least impacted by SCTLD (Truc et al. 2023). Unfortunately, UV treatment of ballast water did not reduce the potential of transmission (Studivan et al. 2022). Poor water quality around residential areas could also promote stress and disease sensitivity in corals (Truc et al. 2023). As this disease does not seem to be temporally or spatially restricted, SCTLD represents a severe threat for coral reefs (Brandt et al. 2021). Without high-level hygiene regulations, there is a high risk that it will eventually spread to Pacific reefs (Studivan et al. 2022), further endangering these vulnerable reefs.

Diseases and pathogens can also be spread through migrating plastic debris (Lamb et al. 2018). Since their recent mass production and unsupervised disposal, plastics accumulating on the oceans and beaches have become a major threat to all marine wildlife, including corals (Allen et al. 2017; Lamb et al. 2018). Microplastics can bioaccumulate in various marine organisms and have toxic and lethal effects (Ganesh Kumar et al. 2020), and even corals have been seen ingesting plastics (Hall et al. 2015; Allen et al. 2017) and suffering from bleaching and tissue necrosis when in contact with microplastics (Reichert et al. 2018). Large plastic objects can block sunlight, and damage corals through direct entanglement, or through wave action. Additionally to physical damage, there is an increased disease prevalence in reefs that are in contact with plastic debris (Lamb et al. 2018). Thus, aiming for an improved waste management is critical for improving the resilience of coral reefs (Lamb et al. 2018).

### *Climate change*

When compared to other threats described above, climate change is usually considered the number one threat to coral reefs (Hughes et al. 2017a; Hoegh-Guldberg et al. 2018; Eakin et al. 2022). Global warming can be directly linked to our greenhouse gas (GHG) emissions from burning fossil fuel and land use, efficiently strengthening the greenhouse effect around the earth (Ring et al. 2012). Since the industrial revolution, our GHG emissions have not ceased to increase, and they now reach 55 billion tons of equivalent $CO_2$ per year, thus about 6.8 tons per year per capita (Jones et al. 2023). The sectors producing the most GHGs are energy production (34%), industry (24%), food production and land use (21%), and transport (14%) (Lamb et al. 2021). To limit devastating consequences of climate change on humans, such as extreme droughts or deadly heat waves (Hoegh-Guldberg et al. 2018), major adjustments need to be implemented. While renewable energies need to gradually replace fossil fuels in electricity and heat production (Lamb et al. 2021), over-consumerism should be discouraged, and the number of airplane trips drastically reduced. Unfortunately, a system based on ever-increasing growth and profit renders a reduction in consumption very difficult. A significant progress could lie in the reform of food production, with a reduction of animal agriculture in favor of plant-based protein, which shows drastically reduced GHG emissions (Xu et al. 2021). For instance, as agriculture uses half of the world's habitable land, livestock grazing and feed

production is using 80% of this surface (Ritchie & Roser 2019), which is in turn responsible for deforestation, habitat destruction and mass extinction (Machovina et al. 2015).

With an ever-increasing quantity of $CO_2$ in the atmosphere, the oceans are becoming more acidic (Hoegh-Guldberg et al. 2018). Ocean waters have already acidified by losing 0.2 pH units since pre-industrial times, and are predicted to decrease in a further 0.2 units by 2100 under RCP4.5 (2°C increase) (Hoegh-Guldberg et al. 2018). This will undoubtedly affect coral reefs in various ways. For instance, acidification seems to increase the competitive potential and allelopathy of macroalgae on corals (Hill & Hoogenboom 2022). Although live corals are relatively well protected from acidity through their tissues and ability to internally regulate pH, they can show reduced growth, skeletal density and survival in acidic conditions, and most importantly recruitment rates are strongly reduced (Hill & Hoogenboom 2022). This is likely linked to reductions in CCA cover and altered chemical cues of these algae, which typically serve as settlement inducers (Hill & Hoogenboom 2022). Additionally, dead or partially damaged corals lack a protective tissue layer, and when subjected to acidic conditions, become less dense and significantly more fragile (Hennige et al. 2015). This can have worrying consequences on barrier reefs that are mostly composed of dead corals. These skeletons still serve as effective growing frameworks for other reef organisms and essential breakwaters for costal habitations, but could lose this crucial function as the environment becomes more acidic.

As a consequence of global warming and a disturbed climate, the intensity of tropical storms is expected to increase, further endangering the survival of coral reefs (Bender et al. 2010; Cheal et al. 2017). Together with an intensification of natural disasters and heat waves, bleaching events are also predicted to become more frequent and more severe as climate change goes on (Hughes et al. 2017b). As mentioned earlier, bleaching events, currently the deadliest and most serious threat for corals, are caused by a prolonged and anormal rise in ocean temperatures (Coles & Brown 2003; Eakin et al. 2022). Already in the early 1990's, Peter Glynn made the connection between bleaching events and rising ocean temperatures, and predicted that the projected 2°C increase in temperature would not allow corals to adapt fast enough to survive (Glynn 1993). Based on our current GHG emissions trajectory, coral reefs are predicted to decline almost entirely by 2050 (Hoegh-Guldberg et al. 2007). With all Indo-Pacific coral reefs on maximum bleaching alert level in March and April 2024 (NOAA 2024), the future of coral reefs is looking bleak. A growing number of climate refugees are expected to seek asylum on neighboring islands, because of many coral islands becoming uninhabitable (Connell 2016, 2021). As a consequence, downscaling our reliance on fossil fuel is essential if we want to continue benefitting from the numerous ecosystem services provided by coral reefs (Woodhead et al. 2019).

## 4. Restoration techniques

With growing pressures threatening coral reefs worldwide, there is an urgent need to strengthen the resilience of coral reefs, meaning their capacity to withstand or recover quickly from stressful events. Human interventions aimed at increasing coral abundance and resilience are becoming more elaborate and widespread. They can be considered as temporary palliative efforts to counteract the coral crisis. In fact, the actual long-term benefits of coral restoration against the threat of climate change remain hypothetical (Hughes et al. 2023). Restoration techniques are nowadays incredibly varied, ranging from simple transplantation efforts all the way to large-scale larval seeding projects (Doropoulos et al. 2019a). Coral reef resilience can be enhanced using passive human intervention, through the implementation of marine reserves and regulation of fisheries, or active measures whereby humans directly manipulate the dynamics of degraded reef systems (e.g., coral propagation, artificial reefs, ecoengineering) (Rinkevich 2008; Boström-Einarsson et al. 2020a; Hein et al. 2020; Airoldi et al. 2021) (Fig. 1.1). Active restoration typically involves labor-intensive and small-scale transplantation of coral fragments raised in nurseries (Shafir et al. 2006b). However, considering global climate change, large-scale restoration techniques, such as mass seeding approaches using wild (Doropoulos et al. 2019a; Suzuki et al. 2020) or laboratory raised coral larvae (Edwards et al. 2015; dela Cruz & Harrison 2017; Chamberland et al. 2017), could potentially complement proactive management interventions. While the most used metrics for restoration success are coral growth and survival, almost no studies report the reproductive output of restored fragments, or the potential socioeconomic benefits, such as new employment in coastal communities (Hein et al. 2017; Boström-Einarsson et al. 2020a).

### Coral gardening

Coral fragments used in restoration projects can be harvested from wild donor colonies and directly transplanted. However, this harms donor colonies and reduces the fitness of wild populations. Thus, raising and multiplying coral fragments in a separate coral nursery setting is a more sustainable method to produce corals for restoration projects. Currently, two thirds (68%) of coral restoration efforts involve coral gardening and transplantation (Boström-Einarsson et al. 2020a), where asexually propagated fragments are grown in nurseries before being outplanted to degraded reefs (Rinkevich 1995; Clark & Edwards 1995; Bowden-Kerby 1997). Small coral fragments can be advantageous because they show a proportionally faster growth rate than large fragments (Yap et al. 1998; Lirman et al. 2014; Sam et al. 2021), but they can display a lower survival chance than larger fragments once outplanted (Raymundo & Maypa 2004). Thus, a nursery phase of 4-12 months, depending on the growth rate and initial size of the fragment, is recommended for corals to reach a size where they become less vulnerable for transplantation (Shafir et al. 2006b; Dela Cruz et al. 2015), which is around 10 cm for branching species and 5 cm for encrusting species (Shafir et al. 2010). The variability of the nursery success can be modulated by the diversity of the designs, from cinder block tables

(Herlan & Lirman 2008), plastic tables (Schmidt-Roach et al. 2023), mid-water floating nurseries (Shafir & Rinkevich 2010), suspended frames (Maneval et al. 2021) to rope nurseries (Dehnert et al. 2023). Some designs, such as the suspended nurseries can potentially offer better protection against crown of thorn invasions (Suzuki 2021) and a faster growth rate than fragments held on a solid substrate (Lirman et al. 2014; O'Donnell et al. 2017; Afiq-Rosli et al. 2017), probably through a trade-off for reduced skeletal density (Kuffner et al. 2017), a reduction of sedimentation and competitors (Rinkevich 2014), and an improved water and nutrient fluxes (Shafir et al. 2006a). In addition, other organisms such as mobile invertebrates can use nurseries as habitats (Wee et al. 2019), and mid-water coral nurseries can be considered new and rich miniature ecosystems (Shafir & Rinkevich 2010).

As of 2012, coral gardening was ranked as the most effective method of reef restoration by restoration practitioners (Young et al. 2012). Coral gardening has been practiced since the late 1980's (Guzmán 1991; Rinkevich 1995; Bowden-Kerby 1997), and has now spread to virtually every coral reef region. The Coral Restoration Consortium currently lists 689 coral restoration projects within 255 organizations worldwide (CRC 2023). While projects are spread out globally in 60 countries, about 40% of projects are conducted in only four countries: the USA, Philippines, Indonesia and Thailand (Boström-Einarsson et al. 2020a), so there is a bias towards these regions in published results. Florida also has the most unpublished private, NGO and tourism-based restoration projects (Ferse et al. 2021). Regions such as the southern Atlantic, the southern Indian Ocean or the southern Pacific are still relatively less studied.

Currently, around 59% of nursery studies use branching coral species, especially the threatened Caribbean *A. cervicornis* (Boström-Einarsson et al. 2020b, 2020a), and Pocilloporids *Pocillopora damicornis* and *Stylophora pistillata* (Barton et al. 2015). While 159 other species from 57 genera have been used in various nursery projects, these are still underrepresented relative to the three most common species (Boström-Einarsson et al. 2020b, 2020a). Indeed, as abundant results are available concerning the nursery rearing of *A. cervicornis* for example, these might not be applicable to different species.

Coral nurseries are considered an efficient tool for asexually multiplying coral fragments (Soong & Chen 2003; Barton et al. 2015), with survival reaching 90% and growth averaging 13 cm/year in branching corals after four months (Rinkevich 2000; Shafir et al. 2006a; Lirman et al. 2010). In the nursery phase, massive, tabular and columnar species can show an overall higher survival rate (around 90% survival) than the more sensitive encrusting, branching and foliose morphologies (around 75%) (Boström-Einarsson et al. 2020b, 2020a). Survival can, however, be strongly site-specific and linked to the local predation rate, storm severity or water quality (Young et al. 2012). While survival and growth are the most frequently monitored metrics, most reports do not study survival in relation to the fragments' initial size (Boström-Einarsson et al. 2020a), making success comparison between studies quite complicated. Also, there are more than six different ways to report growth in the published literature (Boström-Einarsson et al. 2020a), which renders comparing restoration outcomes almost impossible. A more unifying monitoring protocol would significantly increase the comparability between

studies. Additionally, other metrics such as the reproductive output and the socioeconomic benefits of restoration can translate the long-term sustainability of a restoration project, but are generally overlooked (Hein et al. 2017)

Indeed, very few experiments have studied the impact of nursery rearing on the reproduction of corals (Amar & Rinkevich 2007; Zayasu & Suzuki 2019). However, it is highly probable that a change in growing substrate and environment would influence all life stages of corals. After being fragmented and depending on their size and timing of fragmentation, corals will generally regress to an immature state, though a few might retain their maturity if fragmented larger than 13 cm (Smith & Hughes 1999), or during late vitellogenesis (Okubo et al. 2007; Rapuano et al. 2023). The stress of fragmentation and change of environment could potentially also limit their future egg size and fecundity (Lirman 2000; Zakai et al. 2000; Kai & Sakai 2008). Their maturity can be disturbed up to three years following fragmentation (Okubo et al. 2007). However, as the growth rate of suspended nursery corals is improved relative to attached corals (Lirman et al. 2014; O'Donnell et al. 2017), nursery corals can potentially reach maturity quicker than wild conspecifics. A nursery setting has proven to be beneficial for the rapid onset of maturity in *A. millepora* or *S. pistillata* (Amar & Rinkevich 2007; Baria et al. 2012). In addition, nurseries can represent highly productive artificial spawning hotspots, thanks to a high density of compatible corals producing significantly more gametes than wild populations (Amar & Rinkevich 2007; Zayasu & Suzuki 2019). If nursery-grown corals are to be used to restore damaged areas and increase the resilience of coral reefs, it is vital that their spawning synchrony and reproductive output are not impaired relative to that of wild corals. As studies comparing the reproductive output of nursery and wild corals are scarce, it is important to improve our understanding of the impact of restoration methods on sexual reproduction.

While fast-growing branching species have always been popular in coral gardening practices, massive species are often left behind, due to their slow growth. Also, encrusting and massive coral morphologies benefit from a solid growing substrate (Forsman et al. 2015) and do not lend themselves to optimal growth when suspended in a nursery. The increased growth of small fragments relative to adult colonies can be used to maximize coral production and to regrow massive corals through micro-fragmentation (Forsman et al. 2015; Knapp et al. 2022). In this process, around 1 $cm^2$ fragments of a single colony are cut with a diamond saw, then glued to a solid substrate at regular distances of around 2 cm (Forsman et al. 2015; Page et al. 2018). Fusion of these fragments in less than a year results in a production of up to 63 $cm^2$ of coral tissue per month (Forsman et al. 2015), which is significantly higher than the 0.5-1 cm yearly growth observed in wild colonies (Madin et al. 2016). As massive coral species are often more resistant to heat stress (Loya et al. 2001) and to COTS predation than branching and tabular species (De'ath & Moran 1998), the restoration of these resistant species could be more cost-effective in the long run than that of less resistant species. Nonetheless, branching and tabular species are indispensable for reef growth, island formation, coastal buffering, and they serve a crucial habitat function for numerous different fish and invertebrate species (Vytopil & Willis 2001; Kerry & Bellwood 2012; Young et al. 2012), that cannot be replicated by

massive species. Also, because of their sensitivity, they are often lacking in damaged environments (Aronson & Precht 2006). In consequence, the enhancement of resistant types of branching or tabular species cover would be beneficial for endangered ecosystems (Bowden-Kerby 2023).

Despite their benefits, coral nurseries are not spared from global and local heat stress (Ladd et al. 2017). As heat stress is to date the biggest threat to corals worldwide (Hughes et al. 2017a), smarter coral nurseries are starting to be developed. These are either focused on growing heat resistant coral genotypes (Morikawa & Palumbi 2019), or protected from bleaching stress through shading (Gantt et al. 2023) or depth adjustment of the nursery (Shafir et al. 2006a). Bleaching is due to a combination of heat and light stress, and recent works demonstrated that reducing light (blue instead of compound light) enhanced coral resistance during heat waves (Gong et al. 2023). There are now projects working on developing shaded nurseries, that should allow protecting coral fragments from heat stress (Hoogenboom et al. 2017; Butcherine et al. 2023) and could thus potentially act as a conservatory for species. Depending on the aim of the nursery, the ideal site will be different. Shallow sites have high light irradiance thus can be beneficial for coral growth (Clark et al. 2022) and for producing large quantities of coral fragments aimed at restoration (Lirman et al. 2010). Other nurseries used for the long-term conservation of coral's genetic diversity might benefit from the more stable conditions of greater depths (Loya et al. 2019), even if the ease of access and growth rates are compromised. Having coral nurseries in different environments could be a way to study physiological acclimatization and to have a backup in case of a mortality event in one of the nurseries. There is, however, still a lack of insight on how the nursery habitat can impact the fitness of corals, and more research would allow improving nursery productivity.

## Coral transplantation

After an initial growing phase in a nursery, coral fragments are generally outplanted. Coral transplantation can become necessary if an area does not receive coral recruits naturally, or if the post-settlement mortality is very high. The aim of transplantation is to increase the reef's resilience and dynamism by introducing missing species or new genotypes with enhanced performances, such as heat or disease resistance. As of 2020, 213 case studies of transplantation projects were summarized in Boström-Einarsson's restoration database (Boström-Einarsson et al. 2020b). Adding to that projects taking place in the last four years and unpublished projects, coral transplantation is quite widespread all over the world (CRC 2023). Branching corals represent the large majority of morphologies used in transplantation studies, with *A. cervicornis*, *P. damicornis* and *S. pistillata* being the most represented species (Boström-Einarsson et al. 2020b, 2020a). Among unpublished private, NGO or tourism restoration projects, 96% reported using branching corals (Ferse et al. 2021).

Methods of attachment of coral fragments vary between transplantation studies: 30% of studies used epoxy, 18% cable ties and only 10% used cement (Boström-Einarsson et al. 2020b,

2020a). Overall these three methods yielded similar survival rates of 73% (Boström-Einarsson et al. 2020b, 2020a). Usually within three months of transplantation, corals begin to cover the attachment substrate with their tissue (Young et al. 2012). Other techniques, such as wedging corals into holes, attaching them to nails, lines, bamboo frames, ropes and mesh are considered much less effective methods by restoration practitioners (Ferse 2010; Young et al. 2012). In the 1990's Smith and Hughes dropped 900 coral fragments on the substrate with no attachment method, and observed low to medium survival and attachment rates, depending on the species and the initial size of the fragment (Smith & Hughes 1999). Larger fragments generally show better survival rates (Smith & Hughes 1999; Soong & Chen 2003; Okubo et al. 2007).

Initial survival and growth can be substantially lower in outplants compared to undisturbed wild colonies or to nursery corals (Lirman et al. 2014), probably due to transplantation and predation stress (Clark & Edwards 1995), and because energy is allocated towards healing and attaching to the substrate (Omori 2019). However, survival rates usually increase in the second year following transplantation (Forrester et al. 2014). Transplant survival can be limited through predation by corallivorous snails, COTS, parrot-, butterfly- and triggerfish (Neudecker 1979; Cabaitan et al. 2015; Shaver et al. 2017), algal competition and strong wave action (Young et al. 2012), and, of course, bleaching events (Fadli et al. 2012). Survival after transplantation reveals a high variability depending on the project, ranging from 43% to 95% after one year in all Caribbean *Acropora* transplantation efforts (Young et al. 2012). Overall, transplantation projects report an increased survival for massive species (73%), followed by encrusting and branching morphologies (65%), tabular (57%), foliose (42%) and finally columnar species (35%) (Boström-Einarsson et al. 2020b, 2020a). As *Acropora* can grow by asexual reproduction through broken branches, their long-term survival can be enhanced by this clonal propagation (Carne et al. 2015). Although fast growth can be an advantage, it can also represent a trade-off in detriment of other energetic costs, for example disease or bleaching resistance (Cornwell et al. 2021; Schlecker et al. 2022). However, as reported trade-offs are extremely variable depending on the studied species and treatments, more research is necessary to establish clear links between corals' vital energetic costs.

As transplantation sites with different environmental gradients influence the outplanted corals' health, these will impact restoration outcomes. However, certain genotypes can respond differently to environmental parameters (Maneval et al. 2021), but the exact causes of their specific responses remain to be explained. The origin of fragments can influence their resistance, for instance transplants from highly variable environments can have a better survival rate (Bay & Palumbi 2017). Their specific symbiotic communities could also allow them to grow faster or better resist heat stress (Jones & Berkelmans 2010). Very few studies compare the genotype effect to environmental effects in transplantation efforts. Planting a large number of different genotypes can be useful in increasing the genetic diversity of a site (Baums et al. 2022), but selecting specific genotypes for their qualities could increase the fitness and resilience of the site. With the threat of climate change, transplanting heat-resistant corals to

improve the resilience of a heat-sensitive reef could be a promising way to prevent widespread bleaching. Studies found that heat resistance can be genetically determined, thus maintained even after transplantation (Barott et al. 2021). In this way, the innate resistance of specific genotypes could enhance the fitness of a sensitive population.

Outplanting coral colonies can benefit a degraded reef by increasing coral cover as well as fish biomass (Dehnert et al. 2023). Coral cover can take years to increase significantly after transplantation (Edwards & Clark 1999). Some transplantation efforts have shown impressive increases in coral cover; *Acropora* fragments transplanted to artificial substrates made of concrete or steel can display up to 64% coral cover in only 2-3 years, rendering the coral cover similar to or higher than reference patches (Fadli et al. 2012; Williams et al. 2019; Knoester et al. 2023). However, if only one genus is transplanted, reference sites will still present a broader coral diversity than restored sites (Knoester et al. 2023). Coral cover increases in transplanted plots can range between 6-23% yearly, and become faster for older plots (Carne et al. 2015). Thus, a long monitoring time could show a gradual improvement in coral cover increase.

Most transplantation studies are monitored for one year or less (Hein et al. 2017), which is not enough time to fully appreciate restoration outcomes. In fact, as corals generally undergo an initial transplantation stress during which growth is reduced (Lirman et al. 2010; Forrester et al. 2014), a longer time frame would better monitor the acclimation and development of restored colonies. A 12 year monitoring for instance shows a very low survival of 9%, but which was not different from the survival of reference colonies (Garrison & Ward 2012). After transplantation, new coral fragments can attract new coral settlers. Sadly, out of 50 unpublished restoration projects, none reported monitoring of the coral recruitment following transplantation (Ferse et al. 2021). Depending on the location and methods, transplantation can show no consistent effect on recruitment rates (Ferse et al. 2013). However, recruitment rates can in some cases be significantly higher at a transplanted site than a healthy reference site, underlining the potential of new outplants in attracting coral larvae (Montoya-Maya et al. 2016).

A general belief is that even if transplantation does not restore large areas of coral reefs, the increased awareness raised by restoration projects is overall beneficial for coral reefs (Ferse et al. 2021). Indeed, public awareness and education are the highest-ranked benefits of coral restoration efforts by restoration practitioners, followed by enhanced fisheries habitat, coral cover, complexity and genetic diversity (Young et al. 2012).

Even if transplantation does not improve coral cover at a large scale, establishing new genetically diverse populations in areas showing a decline of wild population could significantly contribute to species recovery when the new colonies become mature (Baums et al. 2005; Vollmer & Palumbi 2007). Sexual reproduction in outplanted colonies can in fact be a good reef replenishment indicator, but it is often lacking in the typical transplant monitoring results. This is likely a consequence of the short duration of the typical monitoring (Hein et al. 2017). Once transplanted corals become mature, they can insure local long-term larval production

(Doropoulos et al. 2019a). In the few studies that did report reproductive data, reproduction was blocked for most corals 15 months after transplantation, except for a few fragments that were initially over 13 cm (Smith & Hughes 1999), or a few that were both large and fragmented during late vitellogenesis (Okubo et al. 2007, 2009). There is usually a trade-off between growth and reproductive output, where fragments that resorb oocytes show a higher growth rate than those that spawn (Okubo et al. 2005). In other studies, maturity occurred two years after transplantation in *A. cervicornis* (Carne et al. 2015), and after three years in *A. palmata* (Young et al. 2012). Five years after transplantation, *P. verrucosa* direct outplants showed no differences in timing or maturity with wild colonies (Martínez-Castillo et al. 2023). However, nursery-bred outplants can retain a substantially heightened larval production and a higher chance to be mature than wild colonies in the same site, suggesting that the benefit of nursery-rearing can be maintained even seven years after transplantation, likely through epigenetic processes (Horoszowski-Fridman et al. 2020). This underlines the potential of coral transplantation in increasing reef recovery rates, and the importance to monitor the reproductive output of outplants for a sufficiently long duration to assess restoration success.

## Coral restoration using sexual reproduction

While the most common restoration practice is still asexual fragmentation and transplanting of coral fragments, there is a risk that using multiple clones of a few genotypes does not contribute to an improved genetic diversity and thus limits the resistance potential to future perturbations (Baums et al. 2022). Also, transplantation of adult colonies can prove to be very time-consuming for very few surviving colonies, and the substantial number of corals required for restoration could be more easily obtained through sexual reproduction. Thus, restoration practices should also encourage sexual reproduction by growing out corals and not continuously fragmenting below the maturity-size threshold (Kai & Sakai 2008). Ideally, restoration projects should combine asexual and sexual propagation (Randall et al. 2020). Restoration using sexual reproduction has the potential to greatly improve genetic diversity and potential resilience towards future stressors by creating many new genotypes. For endangered species for instance, it is crucial to maintain a healthy genetic diversity ratio (0.5 for *A. palmata* and *A. cervicornis*) (National Marine Fisheries Service 2015), which is the proportion of unique genotypes per number of colonies sampled.

Relative to other coral restoration techniques with 396 records in Boström-Einarsson's database, projects actively using sexual reproduction are largely underrepresented (Boström-Einarsson et al. 2020b, 2020a). There are for instance only 8 restoration studies mentioning larval enhancement, one mentioning sexual propagation and two mentioning sexual reproduction in this database (Boström-Einarsson et al. 2020b, 2020a). Out of unpublished private, NGO and tourism restoration projects, only 6% reported using sexual reproduction to produce corals (Ferse et al. 2021), potentially because it is more labor-intensive and requires more specific techniques than asexual restoration (Rinkevich 1995; Omori 2019).

Transplantation of sexually obtained corals started to be studied in Okinawa and the Philippines and showed high initial survival rates (Omori et al. 2008; Nakamura et al. 2011; Villanueva et al. 2012), and matured after only three or four years (Iwao et al. 2010; Baria et al. 2012). As *Acropora* ex situ reproduction is relatively straightforward and they show a fast growth, they represent the large majority of species used in sexual restoration. Brooding Pocilloporids are also used (Linden et al. 2019), as many of them release larvae on a monthly basis. However, other species are largely underrepresented, either because they produce very little larvae, are harder to maintain and breed ex situ or have a slower growth (Omori 2019).

The reproductive strategy of many coral species is to produce a large number of offspring to counteract predation, in order to allow only a few to survive and recruit (Ramirez Llodra 2002). A single coral colony can produce huge quantities of gametes, up to 260 oocytes per $cm^2$ of coral tissue (Wallace 1985). Thus, once ex situ spawning or in situ collection with floating nets is mastered (Linden et al. 2019; Suzuki et al. 2020), obtaining large quantities of coral spawn slick is relatively uncomplicated. Breeding corals in laboratory settings has been implemented since the 1980's (Shlesinger & Loya 1985; Oliver & Babcock 1992). A laboratory setting has the advantage of removing predation from fish and crustaceans on coral eggs during spawning, thus potentially producing significantly more viable larvae than in situ. Once fertilized and ready to settle, coral larvae can be used to either seed specific artificial substrates, certain degraded areas, or a reef where natural settlement is low (Omori & Iwao 2014).

Artificial substrates can display all shapes or materials, varying from simple push mounts (Boch & Morse 2012), ceramic lattices (Nakamura et al. 2011), clay tripods (Chamberland et al. 2015) or tetrapods (Chamberland et al. 2017). Ex situ rearing of coral recruits can be more controlled, and sheltered from predators and competitors; however, access to symbionts and food should be insured (Joseph Pollock et al. 2017; Huffmyer et al. 2021). After surviving the most sensitive first weeks (Calle-Triviño et al. 2018), recruits can be outplanted or simply seeded on reefs (Chamberland et al. 2017). Survival rates of recruits are generally less than 10% after five months (Epstein et al. 2001; Raymundo & Maypa 2004; dela Cruz & Harrison 2017). Often, cages or refuges are essential to protect small recruits from predation (Nakamura et al. 2011). Although small recruits are very vulnerable (Raymundo & Maypa 2004; Nakamura et al. 2011), they can show significantly higher survival rates in natural conditions even at only one month old than in land-based nurseries with inadequate conditions (Chamberland et al. 2015). Despite the high initial mortality, long-term survival rates of sexual propagules can later surpass that of asexual fragments, making this technique four times as cost-effective as asexual transplantation (Baria-Rodriguez et al. 2019). The seeding of coral recruits also has the potential to accelerate natural recovery across larger areas than transplantation of adults.

Direct in situ larval seeding or larval enhancement is the practice of helping coral larvae to settle at one precise spot. This assisted mass settlement of coral larvae can significantly increase recruitment rates of degraded sites (dela Cruz & Harrison 2017, 2020; Harrison et al. 2021). However, over the long-term, due to high post-settlement mortality, the effectiveness of larval seeding may become negligible next to naturally occurring recruitment (Edwards et

al. 2015). In situ larval enhancement has only been tested in nine studies (Heyward et al. 2002; Omori et al. 2003; Suzuki et al. 2011; Edwards et al. 2015; Cooper et al. 2014; dela Cruz & Harrison 2017, 2020; Cameron & Harrison 2020; Harrison et al. 2021), in Australia, Japan, Palau, Florida and the Philippines. This is thus a relatively unexplored restoration technique. By bypassing the settlement and recruit phase in land-based aquariums, direct larval seeding could be even faster and more cost-effective than recruit outplanting. With constantly improving seeding techniques, hundreds of mature colonies can be produced after just three years (Harrison et al. 2021). Larval seeding could be a scalable way to restore large areas by transporting coral slick by boat to heavily degraded reefs, and thus more cost-effective than transplanting adult colonies (Doropoulos et al. 2019a, 2019b).

The outcomes of sexual restoration could be further improved by new complementary techniques, which are still at the experimental stage. With a careful consideration of ethics and potential risks, human-assisted evolution has been suggested as a way to increase coral's heat tolerance, not through direct genetic modifications but rather with acclimatization through epigenetic mechanisms, selective breeding or the manipulation of the corals' microbiome and algal symbionts through inoculation (Van Oppen et al. 2015). Breeding parents can be selected to produce the desired type of offspring. Crossing of heat-resistant populations could for instance result in heat-resistant offspring (Quigley et al. 2020; Howells et al. 2021). For example, colonies from the hottest reefs in the world, when crossed with naïve colonies, can transfer their heat resistance to their hybrid offspring (Howells et al. 2021). Also, interspecific hybridization could be a way create new heat resistant genotypes (Chan et al. 2018). While high temperatures strongly reduce coral's fertilization chance, thermal priming or the pre-exposure of sperm and oocytes to 30°C for as little as 30 minutes has been shown to increase their fertilization rates at high temperatures significantly (Puisay et al. 2023). The manipulation of symbionts, by forcing the symbiosis of coral recruits with certain strains has recently been experimented: while growth at ambient temperature is fastest in association with *Cladocopium goreaui*, *Durusdinium trenchii* allows for a better growth under heat stress (Quigley et al. 2020). Laboratory heat-evolved symbionts can even be used to further increase bleaching resistance, significantly more than wild strains (Quigley et al. 2023). The artificial infection with beneficial bacterial communities can also be a way to increase bleaching resistance (Rosado et al. 2019). While assisted evolution is still at the experimental stage, a lot more research needs to be implemented before it can be used in restoration efforts.

By crossing specific pairs of parents, selective breeding could produce offspring with desired qualities, such as heat resistance (Quigley et al. 2020; Howells et al. 2021). Selective breeding can produce different fertilization rates based on the compatibility of crossed corals. Varying breeding compatibilities have been observed within all *Acropora* species, but also in *Montipora* and *Platygyra* (Willis et al. 1997). Like many corals, *Acropora* are hermaphrodite but usually self-incompatible (Heyward & Babcock 1986; Willis et al. 1997; Fogarty et al. 2012), thus finding a compatible sexual partner is crucial for fertilization outcome. Significantly different fertilization successes between pairwise crosses have been observed in *A. palmata*

(Miller et al. 2018) and *A. cervicornis* (Koch et al. 2022b). While two-parent crosses usually have lower fertilization rates than batches, some couples can contribute significantly more to batch fertilizations than others (Baums et al. 2013; Iwao et al. 2014). This can be due to the morphology of sperm which can vary between colonies, and cause sperm to swim faster or slower (Baums et al. 2013). Genotype compatibility can result in faster swimming larvae, at ambient or elevated temperatures (Baums et al. 2013), thus offspring with an increased heat resistance. Various experiments have shown strong parental effects in the heat tolerance of the offspring (Dixon et al. 2015; Drury et al. 2021; Johnston et al. 2024). A better understanding of such parental effects is crucial for efforts to optimize offspring survival and fitness. Larvae specifically produced for their enhanced abilities could be used to further improve the success of other restoration practices, such as larval seedings.

Coral restoration projects using sexual reproduction are still very localized and require specific skills and facilities. As they are only tested in certain regions, they are not yet deployable everywhere. For most coral species, sexual reproduction is not yet mastered ex situ. Therefore, available results are still very specific to a few case studies and cannot be generalized everywhere with any species. Despite increasing research in the last decade, there is still a lack of insight on the factors controlling spawning timing and larval dispersal of some coral species (Randall et al. 2020). This is vital to understand for local management purposes, and also if corals are to be bred in laboratory settings. For durable outcomes, restoration efforts should also focus on optimizing recruit growth and survival (Vardi et al. 2021), for instance by providing shelter from predation. Ultimately, interventions to improve the durable coral recruitment in a degraded site could increase the recovery of a reef and its resilience in the face of future perturbations (dela Cruz & Harrison 2017).

## Artificial reefs

If post-settlement mortality is usually high, a method to increase survival would be to offer artificial substrates that are free of competitors and offer shelter from predation. Artificial reefs (ARs) for example are a popular approach for active coral restoration (Clark & Edwards 1994). These artificial structures act as substrates facilitating coral recruitment. Alternatively, they can be used as platforms for the transplantation of coral fragments (Boström-Einarsson et al. 2020a). Once populated by mature corals, they can further enhance coral recruitment via sexual or asexual reproduction or by producing favorable settlement cues attracting new coral larvae (Edwards & Clark 1999; Gleason et al. 2009; Shaish et al. 2010). Also, floating ARs can attract biodiversity to an empty water column and simultaneously benefit from an improved water quality (Baer et al. 2023). In areas where only sand or coral rubble is present due to anthropogenic degradations, artificial structures can help with substrate stabilization and enhance the recolonization by sessile organisms (Boström-Einarsson et al. 2020a).

Originally, AR deployments were mostly ship groundings, then sunken structures that aimed to increase the abundance of commercially targeted fish species (Higgins et al. 2022).

Following the first major bleaching event of 1998, concern for coral's conservation rose and deployment of ARs increased dramatically in the early 2000's (Higgins et al. 2022). As of 2020, there were 4088 published papers mentioning ARs or a synonym in their title, abstract or key, of which 802 papers specifically studied ARs in coral reef ecosystems (Higgins et al. 2022). While the largest AR deployment project was in India by deploying 10,600 modules to restore 3.15 ha of an island from erosion (Jayanthi et al. 2020), Florida is the location with the most AR projects to date, with 42% of scientific experiments on ARs based in the tropical Atlantic (Higgins et al. 2022). In the Pacific, most ARs are deployed in Australia and Indonesia (Higgins et al. 2022; Bracho-Villavicencio et al. 2023). Among ARs built for conservation purposes, only 27% are specifically aimed at increasing the coral cover, while the rest are mainly aimed at increasing fish abundance and habitat quantity (Higgins et al. 2022). Using restoration to create new coral nurseries is a relatively recent concept (Shaish et al. 2008).

Corals are nowadays monitored in about 53% of ARs projects aimed at conservation (Higgins et al. 2022). *Acropora*, *Porites* and *Pocillopora* remain the most frequently reported genera on ARs (Boström-Einarsson et al. 2020b, 2020a). Often, long-term data on survival of species populating artificial versus natural reefs are lacking (Pickering et al. 1999; Brickhill et al. 2005). In fact, most AR studies are monitored for less than two years (Boström-Einarsson et al. 2020a; Bracho-Villavicencio et al. 2023). As changes in coral population dynamics sometimes take decades to establish (Palandro et al. 2008; Baker et al. 2008), AR studies would benefit from a long-term monitoring to distinguish meaningful impacts on coral populations. Long-term monitoring is essential to fully appreciate the evolution of sessile communities and the plethora of different species of mobile fauna using ARs for shelter or feeding (Ng et al. 2017).

Within the different aims of restoration, AR projects aimed at increasing coral cover report a high success rate of 71% (Higgins et al. 2022). Hard coral cover on artificial structures can vary strongly between studied sites, ranging from 2 to 40% after ten years, with many sexually mature colonies, next to an abundant cover of soft corals, gorgonians, anemones, hydroids or zoanthids (Ng et al. 2017). On concrete ARs with added *Acropora* fragments, recruit density was 53 recruits/m² after only one year relative to 31 recruits/m² on natural substrate (Fadli et al. 2012). Although coral recruitment can seem high at the start of AR deployment, survival usually gradually decreases as competing organisms overgrow coral recruits (Hylkema et al. 2023). The survival rates can vary from 0 to 47% between recruits having sizes of 3 mm and 30 mm respectively, confirming the need to protect small recruits from predation until they reach a size-escape threshold, for example above 3 mm (Raymundo & Maypa 2004; Doropoulos et al. 2012) or 9-10 months (Davies et al. 2013; dela Cruz & Harrison 2017). After that, the average survival of corals in AR studies is reported around 66% (Boström-Einarsson et al. 2020b, 2020a). While ARs have shown successful results in coral cover increase, none achieved the same species similarity as adjacent natural reefs (Bracho-Villavicencio et al. 2023), probably due to their different orientation and material composition.

ARs display various shapes, with cubic and pyramidal being predominant (Bracho-Villavicencio et al. 2023), but many other innovative shapes being tested such as steel "spiders" (Williams

et al. 2019), pierced domes or layered discs, which did not differ in coral recruit density, survival or growth (Hylkema et al. 2023). ARs deployed in experimental studies are usually smaller than 5 m², while ARs built for conservation purposes are often larger than 150 m² (Higgins et al. 2022). Typically, they are formed by massive concrete structures (Boström-Einarsson et al. 2020a): 60% of ARs use concrete, 12% plastics, 9% metal and only 7% rocks (Vivier et al. 2021). Biogenic materials such as shells or coral skeletons are very rarely used for building ARs (Bracho-Villavicencio et al. 2023). However, they would have the advantage of blending in with the initial reef and not introducing harmful materials into the environment.

When deployed as coral recruitment substratum, the success of ARs highly depends on the efficiency of materials and surfaces to encourage the spontaneous settlement of coral larvae, as well as the subsequent survival and growth of coral settlers (Yanovski & Abelson 2019). Physical characteristics influencing recruitment dynamics include the composition of the material, their surface texture, color, chemistry, durability, their size and orientation, and the shelter and shading that they offer (Spieler et al. 2001; Doropoulos et al. 2016; Nozawa et al. 2011). The conditioning of artificial materials is also necessary to develop a biofilm that promotes coral settlement, for instance CCA and its associated bacteria (Schuhmacher 1977; Morse et al. 1996; Heyward & Negri 1999). Also, older biofilm shows improved results relative to younger biofilm (Webster et al. 2004; Guest et al. 2010). However, there is still a lack of understanding regarding the best physical parameters of recruitment substrates used in reef restoration (Spieler et al. 2001; Randall et al. 2020).

Various artificial materials have been tested as coral recruitment substrates. Traditionally, settlement studies use unglazed ceramic tiles, which are easily sourced and effective at attracting coral larvae (Harriott & Fisk 1987). More recently, a large panel of materials have been tested, such as polyvinyl chloride (PVC) (Mallela et al. 2017), rubber (Reyes & Yap 2001), brick tiles (Field et al. 2007), glass tiles (Lei et al. 2021), concrete (Burt et al. 2009), petri dishes (Harriott & Fisk 1987), aragonite plugs (Rodd et al. 2022), fiberglass (Loh et al. 2006), metal (Fitzhardinge & Bailey-Brock 1989), clay (Chamberland et al. 2015), 3D printed ceramic (Berman et al. 2023b, 2023a) and 3D printed polylactic acid (PLA) (Ruhl & Dixson 2019; Randall et al. 2021, 2024). Artificial materials can even trigger higher recruitment than on adjacent natural reefs (Chansang et al. 2008). Usually, rougher materials are preferred than smooth ones (Mallela et al. 2017; Mallela 2018). Bioplastics are considered as alternatives to oil-sourced materials to slow the spread of microplastics in the ocean. Furthermore, biomimetics can be used for a more efficient integration of structures in the ecosystem and for increased safety of artificial materials (Fabio et al. 2019; Giraldes et al. 2023). As porous and friable materials such as sandstone are prone to erosion, they show a low recruit survival relative to concrete or ceramic (Burt et al. 2009). This underlines the need to study the durability of a material in seawater before designing ARs.

Few studies have focused on the different durability of artificial materials and their ability to sustain long-term coral recruit survival, likely because of the short monitoring periods (Bracho-Villavicencio et al. 2023). Mortality seems to vary strongly between settlement material,

ranging between 10-87% on concrete, rubber, PVC or metal (Fitzhardinge & Bailey-Brock 1989; Chamberland et al. 2017; Randall et al. 2021). If orientation and shelter availability is similar between materials, their mm-scale texture could influence the survival of coral recruits, by favoring the growth of significantly different fouling organisms (Tebben et al. 2014), that vary in their interactions with coral recruits. In fact, waxed surfaces can increase the survival of coral recruits by preventing fouling by turf algae (Tebben et al. 2014). Certain artificial materials might also attract more fleshy algae (Geraldi et al. 2014), sponges, tunicates (Fitzhardinge & Bailey-Brock 1989) or CCA than others, which can be competitors or inhibitors of coral recruitment. The fouling communities on artificial materials can thus be significantly different from those on natural substrata and closely linked to material characteristics, but the underlying causes are still not fully understood (Svane & Petersen 2001).

As the major factor influencing recruit survival is usually predation (Spieler et al. 2001), shelter or cryptic surfaces on artificial materials are essential for promoting coral recruitment (Price 2010). The micro-complexity of an artificial material, such as added grooves and micro-crevices, can also significantly enhance coral recruitment rates (Nozawa et al. 2011; Edmunds et al. 2014), survivorship and growth (Nozawa 2008; Mallela 2018). Survival is also enhanced on lower sides and on tiles with wider grooves (Randall et al. 2021). These refuges can significantly reduce grazing pressures on coral juveniles (Brandl & Bellwood 2016). Novel technologies such as 3D printing allow for various shapes and textures to be tested as potential AR structures (Ly et al. 2021; Levy et al. 2022; Berman et al. 2023b). For example, 3D printing technology allows one to create and replicate any shape with many different materials, that can be perfectly tailored to fulfill a biological function and serve as potential AR structures (Mohammed 2016; Pioch et al. 2020; Ly et al. 2021). Hole size can show an influence in larval settlement and survival (Whalan et al. 2015). Because of the short duration of most experiments, there is still a knowledge gap on how long-term coral recruitment is affected by material type, size of crevices, and how closely it is linked to the colonizing benthic communities. Poor AR design is the leading cause of projects failing to achieve conservation objectives (Higgins et al. 2022). Therefore, ARs could be more efficiently designed if more research was being conducted on the optimal materials, crevices, and deployment methods.

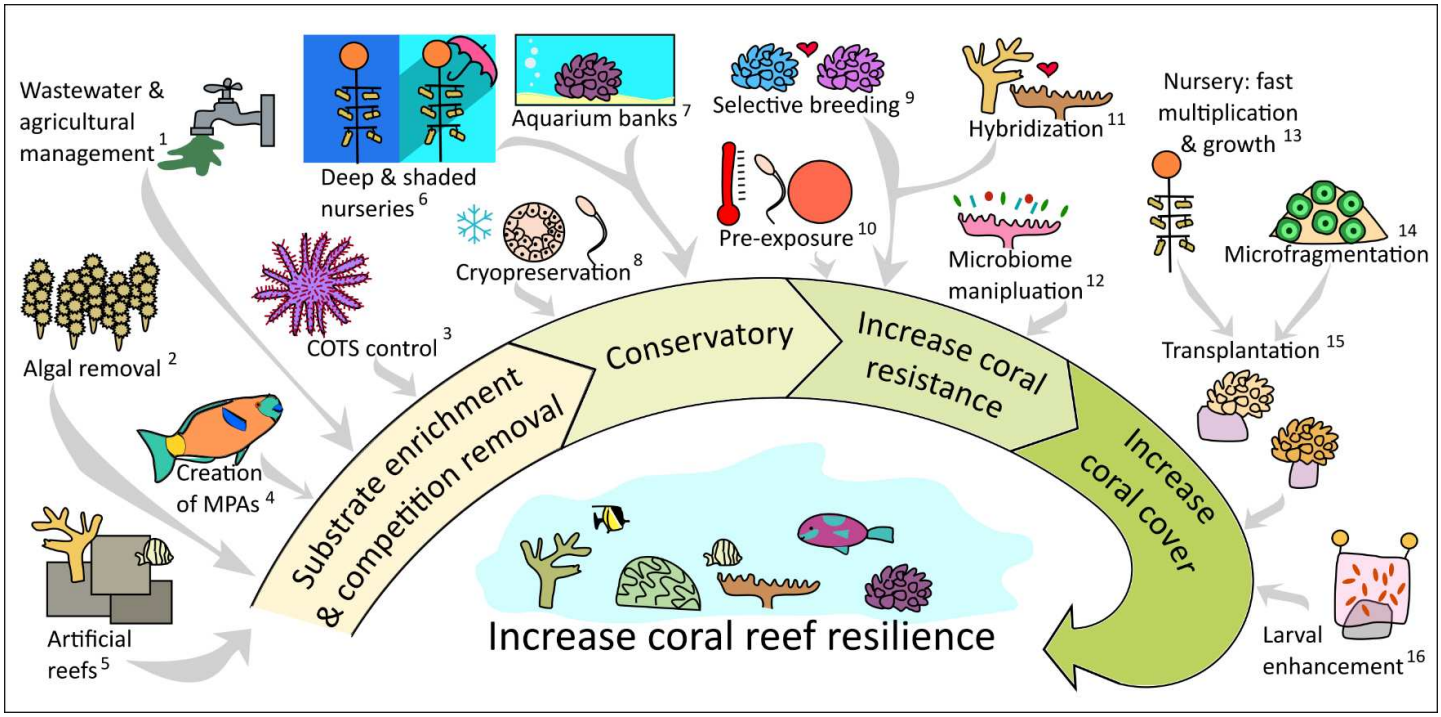



**Figure 1.1.** Main pillars of coral restoration and potential synergy between sectors. Examples of successful implementations: 1 (Wear & Vega Thurber 2015), 2 (Smith et al. 2022), 3 (Westcott et al. 2020), 4 (Selig & Bruno 2010), 5 (Higgins et al. 2022), 6 (Gantt et al. 2023), 7 (da Silva et al. 2019) , 8 (Hagedorn et al. 2019), 9 (Howells et al. 2021), 10 (Puisay et al. 2023), 11 (Chan et al. 2018), 12 (Quigley et al. 2023), 13 (Soong & Chen 2003), 14 (Forsman et al. 2015), 15 (Williams et al. 2019), 16 (dela Cruz & Harrison 2017).

## 5. Thesis aims

Despite the long-term success and ecological relevance of restoration practices still being investigated, the current methods are undoubtedly going to improve in scalability and cost-efficiency with increasing investments in restoration and research projects (Hein et al. 2021). Before investing in large-scale restoration programs, serious feasibility studies need to be undertaken. Major limitations in different steps of coral restoration could be corrected if certain research questions were addressed. The objective of my PhD was to study and evaluate factors influencing the success of different restoration practices trialed in Mo'orea, French Polynesia. The influence of nursery and transplantation habitats on corals' fitness and reproductive capacities, and the characteristics of artificial materials affecting recruit density and survival were investigated. This could aim to better understand limitations associated with restoration practices, then fine-tune and potentially combine certain restoration techniques. This could in turn improve their overall outcome and enhance the resilience of coral reefs in French Polynesia.

Chapter 2 investigates the influence of the nursery habitat, in the lagoon versus the deeper fore reef, on the survival, growth, and reproduction of five key *Acropora* species of Mo'orea

over a year. First, we predicted that survival would remain stable in both habitats. Second, under the assumption that fore reef environment will be characterized by lower seawater temperature and light intensity, we assumed that growth and maturity of coral fragments would be reduced at the fore reef nurseries, due to a lower energy availability. In addition, we investigated in more detail the photophysiological mechanisms of *A. hyacinthus* to better understand how a change in the environment may alter the growth and survival of that species, and provide recommendations for managers.

Once nursery corals reach a certain size, they can be used to improve coral cover in degraded sites through transplantation. Chapter 3 focuses on the transplantation of eight genotypes of nursery-reared *Acropora cytherea* in three different sites of the lagoon in Mo'orea. The symbiont communities of each coral were expected to gradually change in response to transplantation and to be linked to the monitored coral's survival and growth. As the chosen sites differed in their nutrient pollution, coral and algal cover, the transplantation outcome was expected to be improved in the reference site, displaying a high coral cover, relative to the other more anthropized sites with lower coral cover, regardless of the coral's genotype.

If nursery corals are expected to restore degraded sites, then they should be able to breed in synchrony and reproduce just as well as wild populations. Thus, chapter 4 investigates the hypothesis that the reproductive output of nursery-reared corals would be equal relative to that of wild conspecifics. Indeed, corals used for outplanting should restore degraded areas as well as contribute unimpaired to the sexual reproduction of the host population. To combine asexual and sexual restoration practices, we also tested whether fertilization rates could be optimized by selecting specific pairs of parents from the nursery. Then, we hypothesized that we could significantly enhance coral recruitment in degraded areas in the lagoon by encouraging the settlement of larvae obtained during ex situ spawning events. If successful, these techniques could be combined in the future to further improve the outcome of restoration practices.

Finally, in order to better understand the influence of substrates on the first life stages of corals, chapters 5, 6 and 7 explore the potential of innovative materials and shapes in improving natural coral settlement, diversity, survival, and growth. They constitute a collaboration with Seaboost, a company which builds custom and bio-inspired artificial reefs. The first aim was to compare the efficiency of eight innovative and two commonly used control materials as recruitment substrates for corals, and the long-term survival and growth of recruits were monitored. The second aim, detailed in chapter 6, was to test the effect of different degrees of surface complexities and crevice sizes on coral recruitment, recruit size, survival, and growth. The material and complexity's influences on epibenthic communities were also investigated, as well as potential correlations between benthic organisms, recruit density and survival.

# Chapter 2

## Physiological acclimation to nursery habitat variation in South Pacific Staghorn corals

# Physiological acclimation to nursery habitat variation in South Pacific Staghorn corals

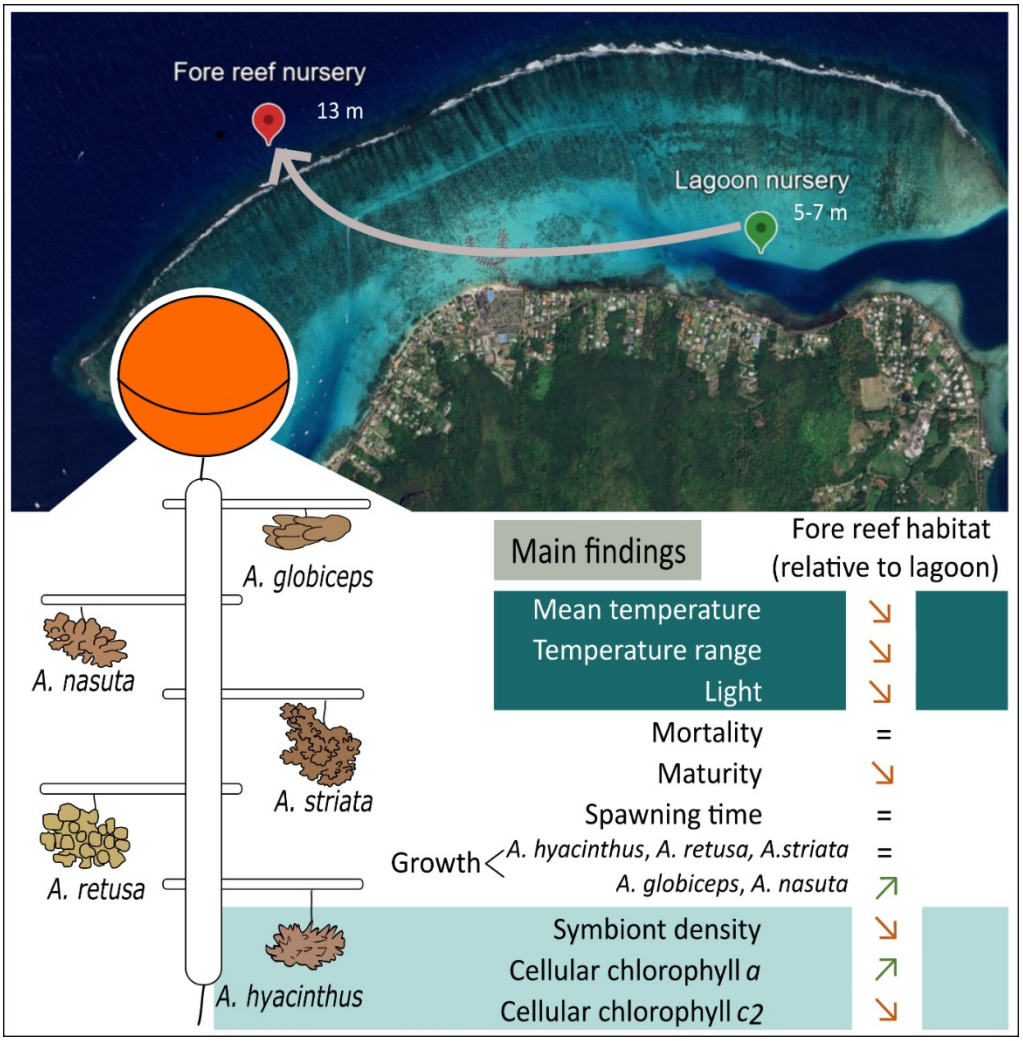
**Running head:** Lagoon and fore reef nurseries

*Camille Leonard[1], Isabelle Davezies[1], Julie Stojakovic[1], Laetitia Hédouin[1,2]*

[1] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, BP 1013, 98729 Papetoai, Mo'orea, French Polynesia

[2] Laboratoire d'Excellence "CORAIL", Papetoai, Mo'orea, French Polynesia

## Graphical abstract



## Abstract

Coral nurseries are an increasingly popular tool in coral restoration to help grow, conserve and select coral fragments before transplantation to a degraded site. The coral nursery's success will depend on multiple factors, such as the selection of donor genotypes and species, fragment sizes, nursery design or environmental habitat. Here, we investigated the influence of the nursery habitat (lagoon versus fore reef), on the survival, growth, and reproduction of five key *Acropora* species of Mo'orea, French Polynesia, over a year. While the overall maturity was reduced following transplantation on the fore reef, survival and spawning timing were equal in both habitats. Coral fragment growth was similar in both habitats for *A. hyacinthus*, *A. retusa* and *A. striata*, but was enhanced on the fore reef for *A. nasuta* and *A. globiceps*. This could be explained by a photo-acclimation, as an increased chlorophyll *a* content per symbiont cell was observed for *A. hyacinthus* on the fore reef. The strongest predictors for growth and maturity patterns were the coral species and fragment size, with smaller fragments growing proportionally faster, and larger fragments having a higher probability to be mature, with a species-specific growth rate and maturity threshold. In conclusion, future projects could benefit from building nurseries in distinct environments as we showed evidence that fore reefs can present favorable conditions without compromising coral fitness. Our work also recommends focusing on fragments smaller than 50 $cm^2$ for the fastest-growing species to optimize productivity like *A. hyacinthus* and *A. nasuta*, which also have the smallest size required to reach maturity. Given the degradation of coral reefs worldwide, their survival and growth during the nursery phase is key to ensure effective restoration outcomes.

# 1. Introduction

Coral reefs are invaluable ecosystems that are facing growing threats, particularly through climate change (Hughes et al. 2018). Downscaling our reliance on fossil fuel is crucial if we want to continue benefitting from the numerous ecosystem services provided by coral reefs (Woodhead et al. 2019). These services include the coastal protection from waves and tsunamis for 500 million people living near reefs, the provisioning of food and materials, the cultural significance and the creation of habitats for the most biodiverse ecosystems currently existing (Woodhead et al. 2019). Despite their crucial importance, coral reefs are now facing unprecedented threats and their future under the predicted carbon emissions scenario looks bleak (Hoegh-Guldberg et al. 2018).

Escalating climate impacts and local pressures on coral reefs are increasingly expanding the development of coral restoration methods, as temporary palliative efforts to counteract the coral crisis. The feasibility of large-scale restoration projects remains to be proven, but numerous innovative concepts are currently being trialed (Rinkevich 2019). As challenges and limitations arise, more research is needed to allow for improvements in different restoration stages (Rinkevich 2021). Among the different key phases of the coral restoration methodology, the nursery phase consists in growing coral fragments within coral nurseries, into suitable sizes before transplantation to restore degraded areas (Lirman et al. 2010). Nurseries are now considered an efficient tool for asexually multiplying coral fragments before out-planting them on degraded areas (Soong & Chen 2003; Barton et al. 2015), with survival reaching 92% and growth rates averaging 13 cm/year in branching corals after only four months (Lirman et al. 2010). The variability of survival and growth is often modulated by the diversity of coral nursery designs, from cinder block tables (Herlan & Lirman 2008), plastic tables (Schmidt-Roach et al. 2023), coral trees (Nedimyer et al. 2011), mid-water floating nurseries (Shafir & Rinkevich 2010), suspended frames (Maneval et al. 2021) to rope nurseries (Dehnert et al. 2023). Some designs, such as the suspended nurseries can potentially offer better protection against crown of thorn invasions (Suzuki 2021) and a higher growth rate than fragments held on a solid substrate (Lirman et al. 2014; O'Donnell et al. 2017).

In addition, it is critical to have coral nurseries implemented in areas less likely to be impacted by local or global stressors (Schopmeyer et al. 2012), such as deeper depths with reduced light, or sheltered fore reefs. Shallow lagoon habitats, characterized by high light irradiance, are generally beneficial for coral growth (Cohen & Dubinsky 2015; Clark et al. 2022), and often selected as nursery habitats with a focus on producing large quantities of coral fragments aimed at restoration (Lirman et al. 2010). Other nurseries used for the long-term conservation of coral's genetic diversity might benefit from the more stable conditions of greater depths or more distant reefs (Loya et al. 2019), even if less accessible and with lower growth rates. Nurseries located on the fore reef could display not only less heat stress periods if they are located below 20m depth (Pérez-Rosales et al. 2021b), but more importantly lower anthropogenic disturbances and algal competition than in the lagoon (Adam et al. 2021). Currently, our understanding of how nursery habitats impact coral fragment fitness remains

relatively unexplored. Among the few data available, Dehnert et al. (2022) showed that the influence of habitat on coral survival and growth is genus-dependent and origin-dependent, with *Acropora* sampled on the fore reef having a higher survival and growth in the fore reef nursery compared to those moved to a lagoon nursery, despite being at the same depth (5 m). By contrast, *Pocillopora* originating from the lagoon displayed the opposite results (Dehnert et al. 2022). Most experiments focused on survival and growth, while changes in reproductive patterns or photophysiology remain poorly understood in coral nurseries.

Depth is expected to have consequences on the physiological capacities of corals, as the increasing depth decreases light availability for corals. As such, photophysiological mechanisms may change along the depth gradient to acclimate to the new environments, characterized by changes in symbiont density or chlorophyll content to maintain coral growth. Nonetheless, works highlighted that, depending on habitats, corals could either be photophysiologically distinct (Ladrière et al. 2014), or similar despite living in distinct depth habitats (Laverick et al. 2019). The translocation of corals to deeper depths usually leads to an increase in chlorophyll content (Falkowski & Dubinsky 1981), and an increase in symbiont densities (Edmunds & Gates 2002; Tamir et al. 2020). However, other studies have found the opposite trend (Bongaerts et al. 2011; Cohen & Dubinsky 2015). Similarly, while some studies observed a decreased growth with increasing depth (Cohen & Dubinsky 2015; Clark et al. 2022), others noted the opposite effect (Torres et al. 2007; Maneval et al. 2021). The contradictory results could depend on the studied species, the study location or other environmental factors than depth.

Aside from the survival and growth of corals, the reproductive output of corals should be considered in restoration projects (Randall et al. 2020). If corals are constantly fragmented below their maturity-size threshold, they will never reach maturity thus fail to contribute to the local larval production (Kai & Sakai 2008; Randall et al. 2020). On the other hand, as a disturbed reproduction can be a sign of stress (Baird et al. 2018; Burt & Bauman 2019), the maturity of a colony can be linked to the colony's fitness. Especially if a nursery is designed to be a long-term conservatory of genetic diversity (Zoccola et al. 2020) or a source of larvae, spawning should be encouraged (Amar & Rinkevich 2007). Floating nurseries can be highly efficient for an accelerated growth, for the maturity onset in *Acropora millepora* (Baria et al. 2012) and for an increased larval production in *Stylophora pistillata* (Amar & Rinkevich 2007), which can be maintained even years after transplantation back to the reef (Horoszowski-Fridman et al. 2020). While some mesophotic corals can display a reduced reproductive output relative to their shallow counterparts (Shlesinger & Loya 2019), and some show no influence of depth on reproductive output (Villinski 2003; Holstein et al. 2016), very few studies investigated the effect of different nursery habitats on corals' maturity.

With the growing degradation of nearshore reefs, lagoon nurseries are likely to be impacted by increased sedimentation and poor water quality (Edinger et al. 1998; Heery et al. 2018). A shift towards more preserved sites might be necessary to maintain sufficient production of coral fragments in nurseries. Nonetheless, implementation of nurseries in fore reef habitats

will definitely require a better understanding on how a change in light and temperature could modulate coral fragment survival, growth and reproduction. Here, we investigated the influence of nursery habitat (lagoon versus fore reef) on the survival, growth, and reproduction of five key *Acropora* species of Mo'orea island over a year, considered depth generalists until 25 m. First, we predicted that survival would remain stable in both habitats. Second, under the assumption that fore reef environments would be characterized by lower seawater temperature and light intensity, we assumed that growth and maturity of coral fragments would be reduced at the fore reef nurseries, due to a lower energy availability. In addition, we investigated in more details the photophysiological mechanisms of *A. hyacinthus* to better understand how a change in the environment may alter the growth and survival of that species, and provide recommendations for managers. Given the devastating impacts of climate change on corals, their survival and growth during the nursery phase and during predicted heat anomalies is key to ensure effective restoration outcomes.

# 2. Materials and Methods

## 2.1 Sites

The coral nurseries are located on the North coast of the island of Mo'orea, French Polynesia. The lagoon nursery, composed of 30 'coral trees', is located in the channel slope between the Hilton hotel and Paopao pass, from 5 to 7 meters depth (17°29'01.1" S 149°50'03.9" W). Each coral tree is composed of hollow 2 m long PVC tubes, with a 50 mm diameter, anchored in the sand with underground anchors (Gripple) and floats vertically with 10 mm nylon boat line and an orange round rigid plastic mooring buoy. The trees were drilled on ten levels, and two 40 cm plastic branches (10 mm diameter) are inserted and attached at each level. Each plastic branch has four holes, one to secure a cable-tie around the tree trunk, and 3 to support suspended coral fragments. Being situated in the channel, the lagoon nursery is subjected to abundant floating macroalgae (*Turbinaria ornata*, *Dictyota* sp. and *Sargassum* sp.) that gets caught in the suspended coral fragments. Consequently, a bimonthly clean-up was organized, where scuba divers removed the macroalgae with gloves, dish brushes, or knives.

The fore reef nursery is located on the fore reef at 13 meters depth (17°28'47.7"S 149°51'08.1"W), outside of Opunohu pass. Holes were drilled into the rock with a Nemo underwater hammer drill, fitted with 10 mm Fischer nylon wall plugs and 25 mm stainless steel ring screws. Trees were anchored by three ring screws with a 10 mm nylon boat line. As herbivory by parrotfish was abundant on the fore reef, no clean-up of macroalgae was necessary.

## 2.2 Environmental parameters

Temperature loggers (HOBO Water Temperature Pro v. 2 Data) and light loggers measuring photosynthetically active radiation (PAR) (DEFI2-L JFE Advantech) were deployed at both sites. Light loggers were fastened upright at 7 and 13 m for 11 days, measuring PAR every 15 minutes.

## 2.3 Corals

Coral fragments were suspended on a nylon fishing line with aluminum barrel sleeve crimps. Five *Acropora* species were used (See photographs in Fig. 2.S1). All five are considered depth generalists, as Madin's coral trait database, the following depth limits are given: *A. hyacinthus*: 0-26 m, *A. globiceps*: 0-20 m, *A. nasuta* 0-30 m, *A. retusa*: 0-29 m and *A. striata*: 10-34 m (Madin et al. 2016), although *A. striata* is also found in lagoons (Wallace 1999). Coral fragments were growing in the lagoon nursery for at least six months before being moved to the fore reef nursery. Because depth of origin was constant for all corals surveyed (around 13 m on the fore reef), it was not used as a factor in our analysis. There were initially three replicates from the same colony of origin in each habitat.

Species identification was done with the colony morphology and the skeletal morphology of bleached branches (Fig. 2.S1). *Acropora globiceps* forms sturdy, up to 25 mm thick, terete branches, with wide open radial corallites, its branches often fused in groups of up to five (Wallace 1999). *Acropora hyacinthus* forms large tables with usually a sturdy stalk, filled with single short regular vertical branches (Wallace 1999). *Acropora nasuta* forms neat corymbose colonies with tapering branches, with evenly sized nariform corallites with rounded to dimidiate (halved) openings (Wallace 1999). *Acropora retusa* forms low sturdy corymbose colonies with short terete branches, and mixed sized radial corallites (Wallace 1999). *Acropora striata* shows an indeterminate growth with clumps of irregular upright hispidose branches, with tubular radial corallites with lipped openings (Wallace 1999).

## 2.4 Experiment 1: Survival, maturity, and growth of *Acropora* fragments between two different environmental nursery conditions.

Our first experiment aimed to determine how the nursery environmental habitat would influence the survival, growth, and maturity of *Acropora* species. Briefly, after fragmenting and growing coral fragments in the lagoon nursery of Mo'orea, French Polynesia, we launched an experiment to assess how a change in coral nursery environment may influence coral fragment fitness in March 2021. To do that, we selected five species of *Acropora* (*A. globiceps, A. hyacinthus, A. nasuta, A. retusa* and *A. striata*) present at the lagoon nursery. For each species, three fragments of 7-27 different colonies of origin were moved from the lagoon (5-7 m) to a nursery (13 m) on the fore reef (n = 200 fragments total), while three fragments from the same colonies of origin were maintained at the lagoon nursery (n = 200 fragments total). For each fragment, the survival was recorded every month, visually and with a picture taken with an Olympus TG3 with an underwater housing.

Maturity was checked a few days following the full moons of September, October, November, and December 2021, by breaking a 2 cm branch off each fragment and checking inside the polyps. Fragments were noted as either empty, with white eggs or with mature eggs if these were pink/reddish pigmented. Split spawning was noted when the same fragment, colony of origin or species was mature on more than one month.

To characterize growth of coral fragments, photos were taken with an Olympus TG3 with an underwater housing, every 3-6 months. The largest surface of the coral was shown on the picture, with a ruler behind the coral for scale, the surface being parallel to the camera. The fisheye distortion was corrected on GIMP (GIMP Development Team 2019) (Main distortion = 37, edge distortion = 2). Size was measured using ImageJ (Schneider et al. 2012). The maximum diameter and the largest surface in $cm^2$ (with the freehand polygon selection tool, around the outline of the largest coral surface) were measured.

Relative growth was expressed similarly to previous studies (Neal et al. 2015; Suggett et al. 2019; Howlett et al. 2021), as a percentage increase per day, calculated as the surface difference (final-initial) divided by the initial surface and the number of days between two

measures. The initial surface of our fragments ranged from 3.3 to 311.0 $cm^2$. The coral size was measured in the lagoon before moving the fragments to the fore reef (March-April 2021), then in both habitats after three months (July 2021), nine months (January 2022) and twelve months (April 2022). The dataset for growth analyses was reduced due to some loss observed, so it was performed on species for which we had at least one fragment out of the three replicates in each habitat after one year.

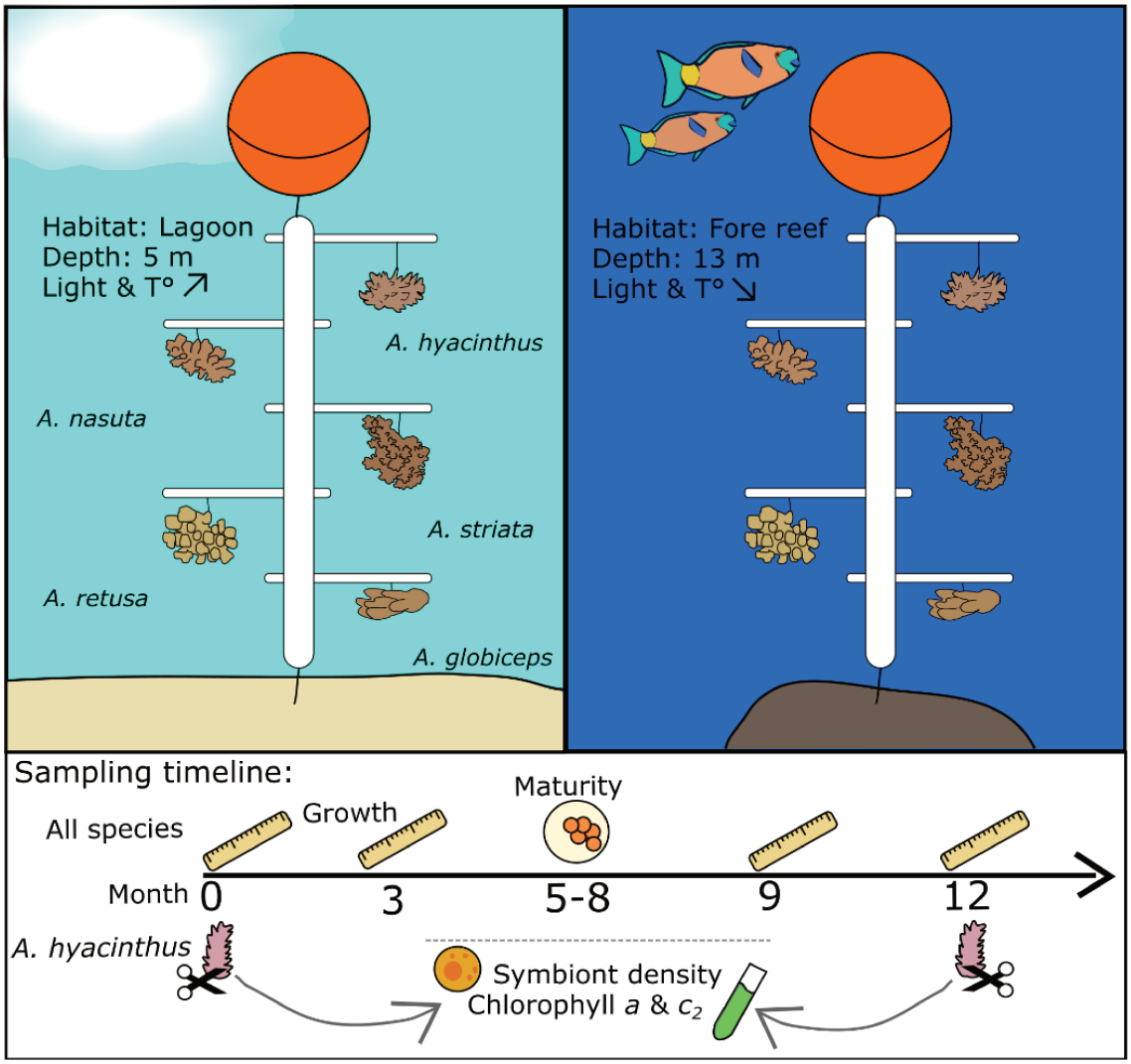



**Figure 2.1.** Simplified setup of nursery trees in lagoon and fore reef habitat (one fragment shown instead of three per branch). Experiment 1 on all species, with punctual photographic surveys to analyze survival and growth, and the survey of maturity. Experiment 2 on *A. hyacinthus* only looking at the photophysiology in both environments.

## 2.5 Experiment 2: Photophysiological response of *A. hyacinthus* fragments between two different environmental nursery conditions

In order to better apprehend biological mechanisms involved in survival and growth of fragments between the two environmental nursery conditions, for *A. hyacinthus*, we investigated its photophysiological response. To do that, before the transplantation of fragments to the fore reef nursery, small samples of *A. hyacinthus* measuring 2-4 cm (from 121 colonies, 26 colonies of origin ) were cut from each coral with plyers, put in clean zip lock bags, transported in ice for 15 min before being stored at -80°C until further analysis. After a year, small samples of all surviving *A. hyacinthus* colonies were collected in the same way in both lagoon and fore reef nurseries.

### 2.5.1 Tissue removal

Each coral sample was retrieved from the -80°C freezer and put in a clean zip lock bag, and the tissue was removed from the skeleton with ice cold filtered seawater (FSW) (Whatman® glass microfiber filters, binder free, Grade GF/C circles, 47 mm diameter and 1.2 µm pores) and a compressed air blow gun. The entire tissue slurry was weighed then homogenized with a Bio-Gen PRO200 Homogenizer for 40 seconds. A 40 mL aliquot was taken and centrifuged at 9000 rpm and 4°C for 20 min. After removing the supernatant, the pellet was resuspended in 3 mL ice cold FSW. Aliquots of 1 mL were kept for the symbiont density and chlorophyll concentration analysis. Coral skeletons were placed in 4° bleach for 24 h, then air dried for 48 h. Afterwards, the skeleton volume and surface of live tissue (total surface - surface of the cut) were recorded from a 3D scan using Exscan S by Shining 3D.

### 2.5.2 Algal endosymbiont density

With a 1 mL aliquot of the symbiont pellet, six independent replicates of cell counts were done using a hemocytometer. Endosymbiont cell density was normalized to the coral's surface area (cells $cm^{-2}$).

### 2.5.3 Chlorophyll *a* and $c_2$ content

1 mL aliquots of the symbiont pellet were vortexed and centrifuged for 5 min at 12,000 rpm. The supernatant was entirely removed, and the pellet resuspended in 1 mL of 100% acetone. The tube was then stored at 4°C in the dark for 24 hours. After being vortexed and centrifuged for 5 min at 12,000 rpm, the supernatant was measured spectrophotometrically (k = 630, 663, and 750 nm) with a BioTek Epoch 2 Microplate Spectrophotometer. Chlorophyll *a* and $c_2$ contents were calculated with the equations from Jeffrey and Humphrey (Jeffrey & Humprey 1975), after subtracting the acetone blank. The pellet was resuspended in 1 mL of 100% acetone, then the same measure was repeated 24 h later and the first and second chlorophyll concentrations were summed. The chlorophyll concentrations were normalized to the coral's surface area ($\mu g.cm^{-2}$) and to endosymbiont cells (pg. $cell^{-1}$).

## 2.6 Statistical analysis

Temperature was analyzed over four months with recordings every 30 minutes, by looking at differences in means, maximums, minimums, and daily range, with a paired Wilcoxon test. PAR was analyzed between 9 am and 3 pm and compared between the two habitats (attenuation, maximum, minimum, daily range), with a paired Wilcoxon test.

Maturity was expressed as the percentage of fragments with mature eggs per colony of origin per species and per habitat. The influence of species, habitat, and size on the presence of mature oocytes were tested with a binomial generalized mixed model (*glmer*) from the *lme4* package (Bates et al. 2015), with the colony of origin as a random factor. Main effects and interactions between species and habitat and size were analyzed with the *Anova* function from the *car* package (Bates et al. 2007). The probability to be mature at certain sizes was explored with the *predict* function on a binomial *glm* with only size as a response variable.

The timing of the maturity was expressed as the mean numeric month of occurrence of mature eggs for each colony of origin separately. It was analyzed with *Anova* on a linear mixed model with the nursery habitat and species as explanatory variables and colony of origin as a random factor. Split spawning was noted when there was more than one month of maturity for a given species or colony of origin.

Coral growth was analyzed with a linear mixed model with a negative binomial distribution (*glmmTMB* function from the *glmmTMB* package) (Brooks et al. 2017), with species (*A. globiceps, A. hyacinthus, A. nasuta, A. retusa* and *A. striata*), habitat (lagoon and fore reef), time (3 growth periods: 0-3 months, 3-9 months, 9-12 months) and the fragment's initial size as explanatory variables and the colony of origin as a random factor.

Photophysiology was analyzed on *Acropora hyacinthus* fragments with linear mixed models (*lmer* function), with the nursery habitat as the explanatory factor and the colony of origin as a random factor. Differences were obtained with the *Anova* function. The linear relationship between *A. hyacinthus* growth and symbiont density was verified with the random distribution of the residuals versus predicted values of coral growth. *A. hyacinthus* growth was analyzed with a linear mixed model with the lowest Akaike information criterion (AIC), with explanatory variables being symbiont density, chlorophyll *a* and $c_2$ per symbiont cell, nursery habitat, original size of the fragment, and colony of origin as a random factor. Main effects and interactions between all variables except size were tested with the *Anova* function.

All analyses were performed in R using RStudio version 2023.12.1. All scripts and raw data are available on GitHub (github.com/CamiLeonard/Nurseries).

# 3. Results

## 3.1 Environmental parameters

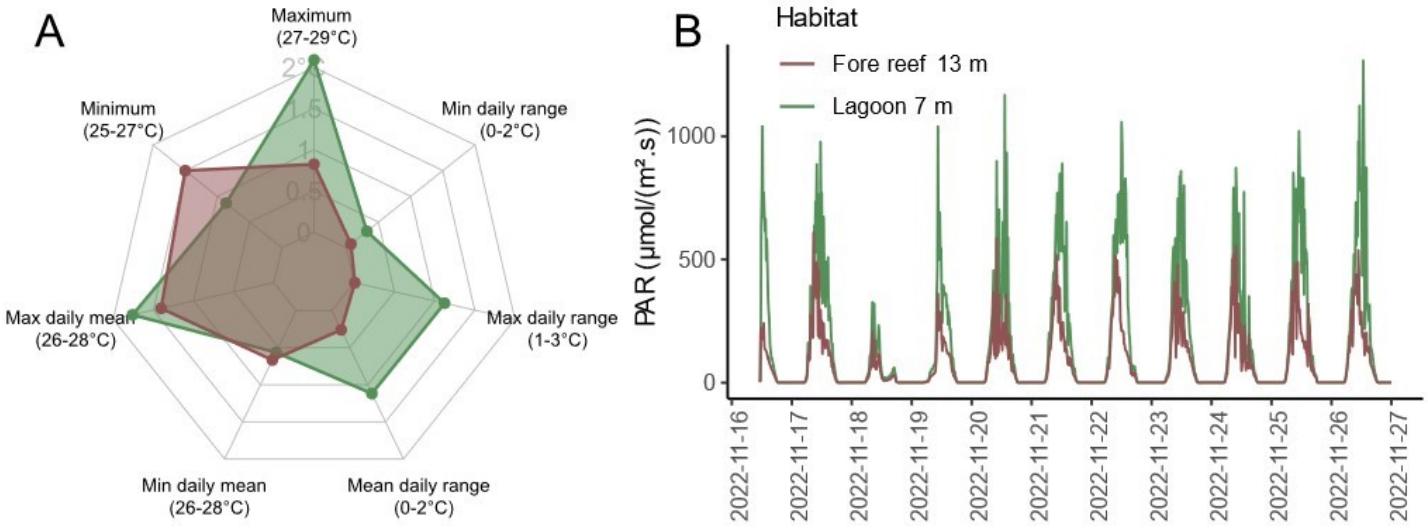



**Figure 2.2.** Temperature variations (A) observed at lagoon (7 m) and fore reef nursery (13 m), maximum, minimum, maximum daily mean, minimum daily mean, mean daily range, maximum daily range and minimum daily range, between July and November 2021. Each axis represents 2°C, with a different scale depending on the measured variable, indicated in parentheses. (B) Photosynthetically active radiation over 11 days at 7 and 13 m depth.

Between July and November 2021, daily mean temperatures, maximums, minimums, and daily ranges in the lagoon (7 m) and on the fore reef (13 m) were significantly different (Fig. 2.2A). Daily maximums in the lagoon were on average 0.67 ± 0.31°C (mean ± SD) warmer in the lagoon compared to 13 m, and minimums were 0.19 ± 0.19°C colder in the lagoon. The daily temperature range was on average 1.12 ± 0.35°C in the lagoon but only 0.26 ± 0.16°C at 13 m.

Photosynthetically active radiation was significantly higher at 7 m than at 13 m ($p < 0.001$), the mean maximum daily PAR being 962.6 ± 249.2 µmol photon $m^{-2}s^{-1}$ at 7 m and 463.8 ± 135.2 µmol photon $m^{-2}s^{-1}$ at 13 m (Fig. 2.2B). Mean PAR between 9 am and 3 pm was 469.5 ± 263.4 µmol photon $m^{-2}s^{-1}$ at 7 m and 207.8 ± 126.5 µmol photon $m^{-2}s^{-1}$ at 13 m. The average light attenuation from 7 to 13 m between 9 am and 3 pm was 52.4 ± 16.8%.

## 3.2 Experiment 1. Survival, maturity and growth of *Acropora* fragments between two different environmental nursery conditions.

### 3.2.1 Mortality

Overall mortality was very low after one year (2.8%) and statistically similar in both habitats (KW p = 0.16). No major bleaching event was noted during the study period (April 2021-April 2022). Mortality in the lagoon was due to spontaneous bleaching and competition with macroalgae. On the fore reef, however, 16.2% of colonies were lost due to strong swell and

material failure (nylon strings and coral trees breaking), but this was not considered as mortality due to the transplantation.

### 3.2.2 Maturity

**Table 2.1.** Anova result of binomial generalized linear mixed model showing variables explaining the fragment's probability to be mature. The random factor is the colony of origin. Underlined names represent homogeneous groups.

| Explanatory variable | Chisq | Df | Pr(>Chisq) | Explanation |
|---|---|---|---|---|
| **Species** | **10.94** | **4** | **0.027** | ***A. retusa* & *A. nasuta* > *A. striata* & *A. globiceps*** |
| | | | | ***A. hyacinthus* > *A. globiceps*** |
| **Habitat** | **6.53** | **1** | **0.011** | **Lagoon > Fore reef** |
| **Size** | **13.60** | **1** | **< 0.001** | **Larger corals > Smaller** |
| Species * Habitat | 1.36 | 4 | 0.851 | |
| **Species * Size** | **9.61** | **4** | **0.048** | |

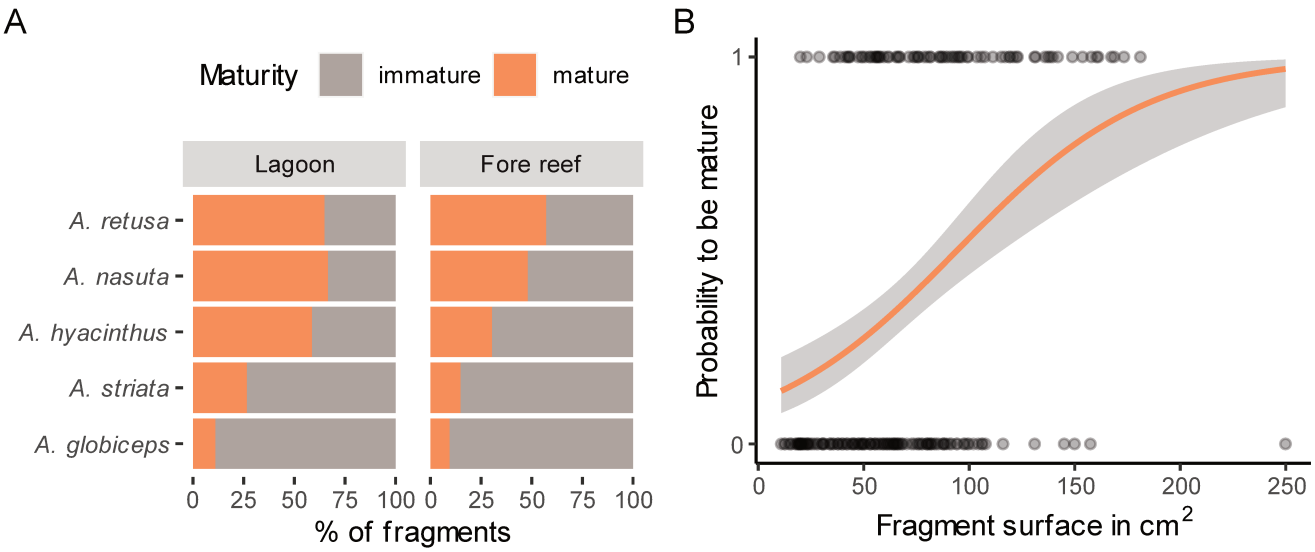



**Figure 2.3.** (A) Percent of mature fragments observed in lagoon and fore reef nurseries, after the full moons of September to November in Mo'orea, French Polynesia. (B) Binomial regression of the probability of nursery fragments to be mature according to their size (surface in $cm^2$, measured in July)

The factor influencing the maturity the most was the size (Table 2.1, Fig. 2.3B). In fact, mature fragments were significantly larger than immature ones (88.6 ± 39.6 $cm^2$ versus 58.3 ± 34.6 $cm^2$), but there was a large variability in size, mature fragments ranging all the way from 19.9 to 181.1 $cm^2$ (Fig. 2.3B). Fragments measuring 50 $cm^2$ had a 27.3% probability to be mature, whereas larger fragments of 200 $cm^2$ had a 91.2% probability to be mature (Fig. 2.3B). Next, the species had a significant influence on the maturity (Table 2.1, Fig. 2.2A); *A. retusa* and *A. nasuta* were more frequently mature than *A. striata* and *A. globiceps*, and *A. hyacinthus* was also more mature than *A. globiceps*. Each species had a different size threshold at maturity (Significant size and species interaction, Table 2.1): for instance, a 100 $cm^2$ fragments of *A. nasuta* had a 97.9% chance to be mature, 89.2% for *A. retusa*, 44.5% for *A. hyacinthus*, 23.6%

for *A. striata* and only 16.4% for *A. globiceps*. Finally, overall lagoon fragments were more likely to be mature than fore reef fragments (Table 2.1), even if the size of fragments during spawning season was the same between habitat (KW pvalue = 0.81), with no significant interactions between species and habitat.

Nursery habitat did not delay in the maturity of fragments, but the month of spawning was dependent of coral species. Mature oocytes were observed in September for 17.1% of *A. striata* fragments, in October for 2.9% of *A. hyacinthus*, 33.3% of *A. nasuta* and 25% of *A. retusa*, in November for 6.7% of *A. globiceps*, 31.6% of *A. hyacinthus*, 18.2% of *A. nasuta,* 35.4% of *A. retusa* and 2.9% of A. striata, and finally in December for 3.3% of *A. globiceps* and 2.6% of *A. hyacinthus*. Split spawning (spawning of a single species over more than one month) was not observed on any single colony (each colony spawned once). However, split spawning was observed in every species except for *A. globiceps*. 29.7% of individual colonies of origin also showed split spawning between their clonal fragments (clones that did not spawn in the same month).

### 3.2.3 Growth

**Table 2.2.** Anova result of generalized linear mixed model showing variables explaining coral growth (Random factor 'colony of origin' accounts for 4.3% of total variance). Underlined names represent homogeneous groups.

| Explanatory variable | Chisq | Df | Pr(>Chisq) | Explanation |
|---|---|---|---|---|
| **Species** | **57.75** | **4** | **< 0.001** | ***A. nasuta* & *A. striata* > *A. hyacinthus* & *A. retusa* > *A. globiceps*** |
| **Site** | **9.39** | **1** | **0.002** | **Fore reef > Lagoon** |
| **Time** | **35.24** | **2** | **< 0.001** | **0-3 > 3-9 & 9-12 months** |
| **Initial size** | **141.21** | **1** | **< 0.001** | **Smaller > Larger corals** |
| **Species * Site** | **12.6** | **4** | **0.013** | ***A. nasuta*: Fore reef > Lagoon**<br>***A. globiceps*: Fore reef > Lagoon**<br>**Lagoon: *A. nasuta* > *A. hyacinthus* & *A. retusa* > *A. globiceps***<br>***A. striata* > *A. hyacinthus* > *A. globiceps***<br>**Fore reef: *A. nasuta* > *A. hyacinthus* & *A. retusa* > *A. globiceps***<br>***A. striata* > *A. globiceps*** |
| **Species * Time** | **42.22** | **8** | **< 0.001** | **0-3 months: *A. nasuta* & *A. striata* > *A. retusa* & *A. hyacinthus* > *A. globiceps***<br>**3-9 months: *A. nasuta* & *A. striata* & *A. retusa* & *A. hyacinthus* > *A. globiceps***<br>**9-12 months: *A. hyacinthus* > *A. globiceps***<br>***A. nasuta*: 0-3 > 3-9 & 9-12 months**<br>***A. striata*: 0-3 > 3-9 & 9-12 months**<br>***A. retusa*: 0-3 > 9-12 months**<br>***A. globiceps*: 0-3 & 9-12 > 3-9 months** |
| Site * Time | 3.55 | 2 | 0.169 | |
| Species * Site * Time | 11 | 8 | 0.202 | |

Overall, the coral species, the nursery habitat, time and the initial size significantly impacted the coral growth (Table 2.2, $p < 0.05$, Fig. 2.S2). The smaller fragments grew significantly faster than the larger ones relative to their initial size (see linear regression in Fig. 2.S2). Fragments that were smaller than 50 $cm^2$ grew on average 147.8 ± 104.8% in a year (12.3% per month), medium fragments 85.9 ± 64.5% and large fragments (bigger than 100 $cm^2$) only 65 ± 51.5% in a year.

*Acropora nasuta* grew the fastest (155.3 ± 104.3% of original size in one year, mean ± SD, Fig. 2.4), followed by *A. striata* (131.6 ± 83% in one year), *A. hyacinthus* (118.5 ± 97% in one year), *A. retusa* (103.1 ± 76.6% in one year), and *A. globiceps* (60.8 ± 46.3% in one year).

There was a significant effect of the habitat, but in interaction with the species (Table 2.2). For *A. nasuta* and *A. globiceps*, growth was 32.5% and 44.9% faster in the fore reef nursery than in the lagoon nursery, respectively. For the other species, there were no significant differences between habitats.

There was also an interaction of the time and the species on coral growth. For *A. nasuta*, *A. striata* and *A. globiceps*, fragments grew on average 59.5% faster 0-4 months than 4-9 months after transplantation and 58.0% faster than 9-12 months (Table 2.2). For *A. retusa*, fragments grew 47.4% faster after 0-3 months than after 9-12 months. For *A. hyacinthus*, there was no significant difference in growth with time.

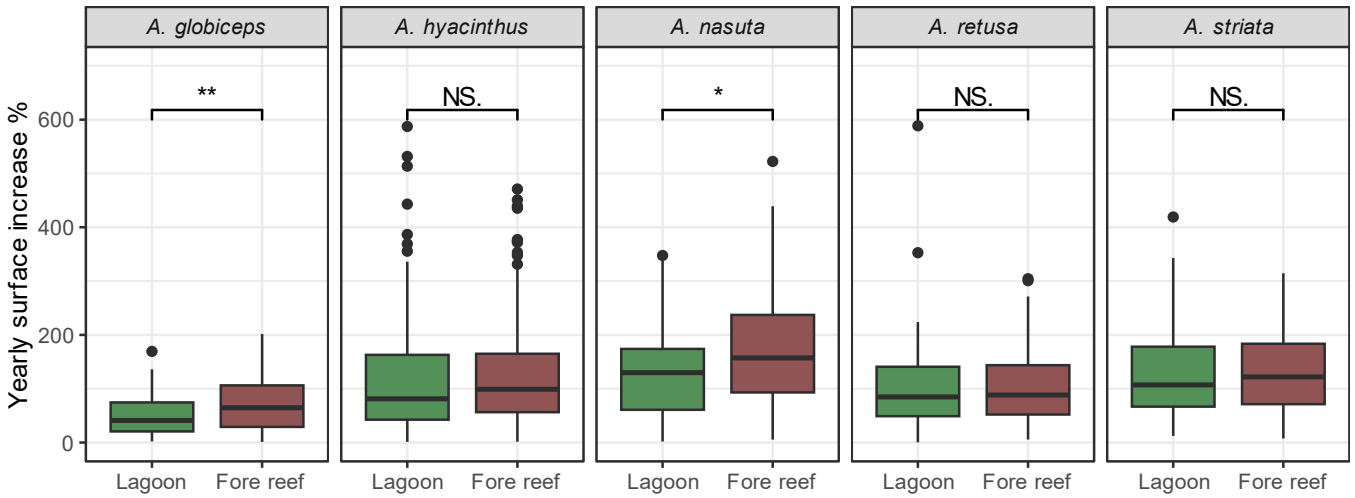



**Figure 2.4.** Percent of yearly surface increase (mean Size difference*100/initial size*number of days *365) of five *Acropora* species growing in suspended nurseries in the lagoon or on the fore reef of Mo'orea.

## 3.3 Experiment 2. Photophysiological response of *Acropora hyacinthus* fragments between two different environmental nursery conditions.

### 3.3.1 Photophysiology

For coral fragments that were maintained in the lagoon nursery, no change in symbiont density (lmer with colony of origin as a random factor, p = 0.23), chlorophyll *a* (p = 0.67) and c2 concentrations (p = 0.08) was noted between the start and the end (one year after) of the experiment However, the photophysiology of coral fragments transplanted to the fore reef nursery showed significant changes. Symbiont density had decreased by 16.8% (Fig. 2.5A, p = 0.01) on the fore reef compared to the lagoon. By contrast, chlorophyll *a* per symbiont cell had increased by 12.3% (Fig. 2.5B, p < 0.001) on the fore reef, and chlorophyll $c_2$ per cell decreased by 25.7% (Fig. 2.5C, p < 0.001).

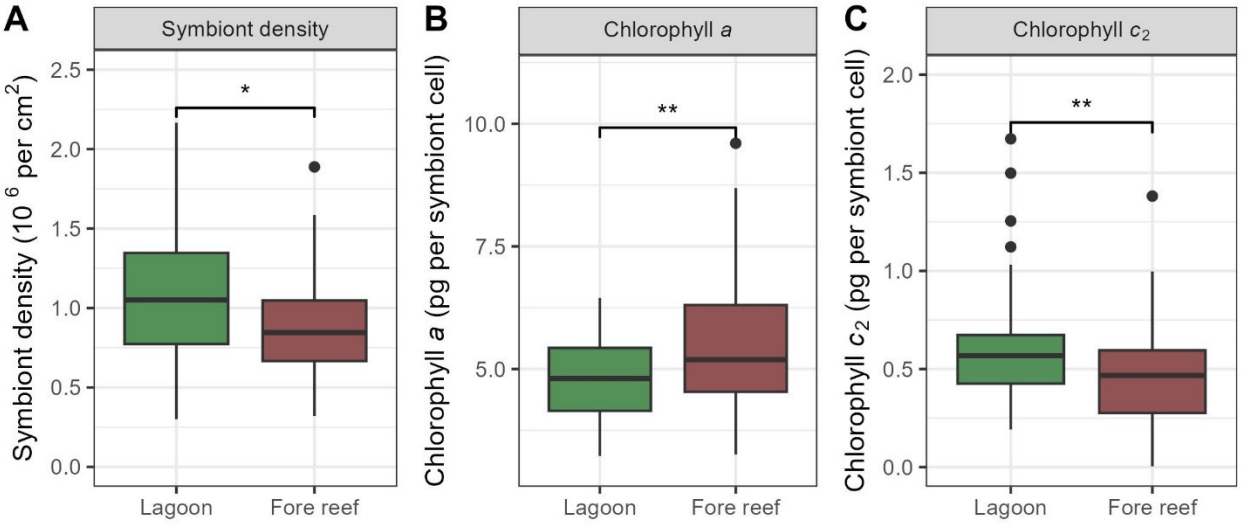



**Figure 2.5.** Photophysiology in *A. hyacinthus* one year after the nursery setup, in lagoon and fore reef habitats (A) Symbiont density (in million cells per $cm^2$ of coral tissue), (B) chlorophyll *a* and (C) chlorophyll $c_2$ concentrations. *****: pvalue < 0.05, ******: pvalue < 0.01

### 3.3.2 Influence of photophysiology on coral growth

The symbiont density had the biggest impact on coral growth. There was a significant positive influence on coral growth and a significant interaction with chlorophyll *a* concentration and the nursery habitat (Table 2.3). On the fore reef nursery, the linear relationship between symbiont density and coral growth was more important than in the lagoon (Fig. 2.6). The size of *A. hyacinthus* corals at the start of the experiment also had a major impact on their growth (Table 2.3). In fact, corals that started off smaller had a much faster growth relative to their size.

**Table 2.3.** Anova result of linear mixed model showing variables explaining one-year coral growth. The random factor colony of origin accounts for 21.2% of the total variance.

| Explanatory variable | Chisq | Df | Pr(>Chisq) |
|---|---|---|---|
| **Symbiont density** | **33.57** | **4** | **< 0.001** |
| Chlorophyll *a* per cell | 5.99 | 4 | 0.2 |
| Chlorophyll $c_2$ per cell | 5.61 | 3 | 0.132 |
| Habitat | 7.97 | 4 | 0.093 |
| **Initial size** | **25.37** | **1** | **< 0.001** |
| **Symbiont density * Chlorophyll *a* per cell** | **13.99** | **2** | **< 0.001** |
| Symbiont density * Chlorophyll $c_2$ per cell | 0.01 | 1 | 0.917 |
| Chlorophyll *a* per cell * Chlorophyll $c_2$ per cell | 0.82 | 1 | 0.365 |
| **Symbiont density * Habitat** | **9.07** | **2** | **0.011** |
| Chlorophyll *a* per cell * Habitat | 3.95 | 2 | 0.139 |
| Chlorophyll $c_2$ per cell * Habitat | 0.2 | 1 | 0.654 |
| Symbiont density * Chlorophyll *a* per cell * Chlorophyll $c_2$ per cell | 1.11 | 1 | 0.293 |
| Symbiont density * Chlorophyll *a* per cell * Habitat | 2.36 | 1 | 0.125 |
| Symbiont density * Chlorophyll $c_2$ per cell * Habitat | 0.21 | 1 | 0.644 |
| Chlorophyll *a* per cell * Chlorophyll $c_2$ per cell * Habitat | 0.57 | 1 | 0.452 |
| Symbiont density * Chloro *a* per cell * Chloro $c_2$ per cell * Habitat | 2.01 | 1 | 0.156 |

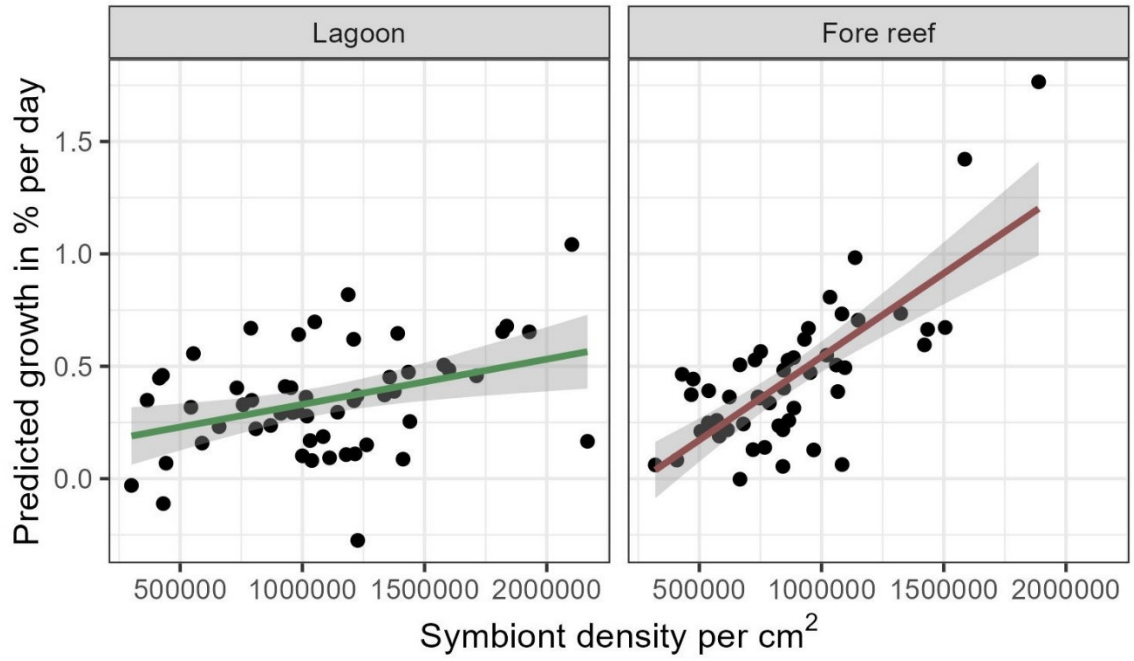



**Figure 2.6.** Linear mixed model predicted growth of *A. hyacinthus* corals in percentage of initial size per day according to their symbiont density per $cm^2$ of coral tissue, with a linear regression and a ribbon showing the 95% confidence interval.

## 4. Discussion

Given the escalating effects of climate change, coral restoration has received an exponential interest this last decade (Newlands 2020). Most restoration projects include a nursery phase that allows coral fragments to grow in a favorable habitat before transplantation to the degraded sites (Soong & Chen 2003). The coral nursery success will depend on multiple biotic and abiotic factors, such as the selection of donor colonies, the design of the nursery, or the nursery's environmental habitat (Maneval et al. 2021). Even if most restoration projects are building coral nurseries in lagoon habitats for practical reasons (Shaish et al. 2008; Barton et al. 2015), other habitats such as fore reefs are becoming of real interest for building coral nurseries or even coral conservatories of genetic diversity at a local scale. In this work, we focused our attention on the role of the nurseries' environmental habitat and the selection of coral species, as these two criteria remain major challenges to ensure success in restoration projects considering the contrasting results reported on the influence of nursery habitats on fragments' fitness. We highlighted that the habitat of the coral nursery (fore reef and lagoon) did not influence survivorship but influenced coral fragment fitness by modulating their maturity, growth and for the case of *A. hyacinthus*, its photophysiological capacity. Also, we reported that the initial size of the coral fragment and the coral species influenced growth and maturity more than the nursery habitat, highlighting that species selection and coral fragment size are key parameters to maximize efficiency of a nursery phase.

The two selected nursery habitats were characterized by distinct light and temperature regimes. The lagoon nursery habitat was characterized by a more variable temperature, with larger daily ranges, higher maximums, and lower minimums, and a higher light availability. This is in accordance with previous studies comparing environmental regimes at different depths in coral reefs with low turbidity (Bongaerts et al. 2011; Ruiz-Diaz et al. 2022). We did not expect an altered coral survival in any nursery habitat, but rather sublethal effects on the overall coral metabolism. We observed a very low mortality (2.8%) of coral fragments after 12 months in both nursery habitats. Our work confirmed the assumption that a change in the environmental regime from a lagoon habitat to a fore reef habitat would not alter survivorship, and also corroborates other studies showing very low mortality despite a depth transplantation (Baker 2001; Cohen & Dubinsky 2015; Clark et al. 2022).

From a physiological point of view, we hypothesized that coral fragment growth and maturity would be reduced on the fore reef nursery, due to a lower light intensity and thus energetic resources being limited. Some populations of deep corals can display a shorter or later spawning season (Prasetia et al. 2017; Feldman et al. 2018) or a reduced coral fecundity and gamete size compared to shallow counterparts (Shlesinger et al. 2018), and some species will show no reproductive trade-offs with depth (Villinski 2003; Holstein et al. 2016). In our case, while the timing of spawning was similar between habitats, our fragments were overall less likely to be mature on the fore reef than in the lagoon. This could be caused by a transplantation stress after the change of nursery habitat. As the growth followed the opposite trend of the maturity for two out of five species, this suggests the existence of a substantial

growth versus sexual reproduction trade-off in the fore reef nursery. Species seemed to allocate fewer resources to reproduction and more to coral growth after transplantation to the fore reef habitat.

Coral growth in both environments harbored two distinct species-specific patterns. We found either no growth rate difference between lagoon and fore reef nursery habitats (for *Acropora hyacinthus*, *A. retusa* and *A. striata*) or an increased growth rate in the fore reef nursery for two species, *A. nasuta* and *A. globiceps.* This suggests that the habitat is a strong species-specific predictor of coral growth, but more generally that the fore reef environmental conditions did not limit coral fitness, as the reduction in light availability (52% attenuation) in fore reef nursery did not inhibit coral growth. This stands in contrast to our initial hypothesis that coral fragment growth rate would be higher in the lagoon habitat, because of the higher energy coming from photosynthesis associated with the higher light intensity, compared to the fore reef habitat. The assumption that light is the only environmental parameter (out of temperature, salinity, water motion and sedimentation) significantly correlated with coral growth at different depths (Yap et al. 1998), has since received a significant amount of interrogation. There is now a wealth of evidence that depth is a mixed environmental variable, tightly linked to decreasing light but also temperature, water movement, nutrients, and suspended sediments, and each of these cofactors can alter the coral health and physiology (Kahng et al. 2019). While many studies reported an enhanced growth rate at shallower sites for *Acropora* (Ruiz-Diaz et al. 2022; Clark et al. 2022) as well as other genera such as *Porites* and *Stylophora* (Yap et al. 1998; Cohen & Dubinsky 2015), other studies found the opposite trend (Torres et al. 2007; Maneval et al. 2021).

A potential explanation of why coral fragments grow faster or at a similar rate in the fore reef and lagoon reef nursery habitat may be that light availability in both nursery habitats did not reach the critical light limitation threshold that would induce a negative effect on coral fitness by altering photosynthetic efficiency. There is, however, no clear threshold for a reduced growth: in fact, once acclimated to the light regime, corals under low light can exhibit the same rate of photosynthesis than corals under high light (Osinga et al. 2011). Thus, the limiting light threshold at which photosynthesis and growth are reduced depends on the environmental conditions of the coral (Osinga et al. 2011). Shallow water *Porites compressa* grow less when exposed to 150 relative to 700 µmol photons $m^{-2}s^{-1}$ (Marubini et al. 2001). In *Acropora verweyi*, calcification was significantly decreased at 200 relative to 400 µmol photons $m^{-2}s^{-1}$ (Reynaud et al. 2004). If 400 µmol photons $m^{-2}s^{-1}$ is the lower threshold for optimal *Acropora* growth, then our fore reef habitat did not show enough light limitation to reduce coral growth. In fact, on the fore reef the irradiance reached over 400 µmol photons $m^{-2}s^{-1}$ almost daily, and *A. globiceps* and *A. nasuta* showed a rapid acclimation to the new environment by increasing their growth already in the first three months after transplantation. It seems thus unlikely that light alone could modulate coral growth, and other biotic or abiotic factors must act in concert with light to drive the coral growth process.

Light availability in the lagoon nursery may also be affected by water clarity due to land-based pollution, which can generate a shift in spectra to less useful wavelengths (towards yellow and green) (Davies-Colley & Close 1990), altering the growth performance of corals. For example, Dehnert et al. (2022) found that reef-reared *Acropora* tend to grow faster in the fore reef than in a lagoon environment, despite no change in depth. If they suggest that the origin of the fragment influenced its physiological response to a new environment, they also highlighted that the fore reef environment is tightly linked with better water quality characterized by less pollution and disease prevalence (Dehnert et al. 2022). Indeed, shallower environments located in the lagoon are generally defined as brighter habitat than deeper environments of the fore reef, but they are also exposed to increased nutrient levels, runoff, sedimentation and rainfall pollution (Dubinsky & Stambler 1996). These factors may alter the transparency of the water and create a shallow environment with a higher level of transparency variability than in the fore reef, which are less subjected to the influence of land-pollution effects (Bell 1992; Crabbe & Carlin 2007).

Changes and variability in light quality in the lagoon nursery may explain the growth rate observed in our study, but other factors should also be taken into consideration. For example, an increased water flow rate on the fore reef could favor fragment growth (Schutter et al. 2010; Boch & Morse 2012), while high temperatures in the lagoon could reduce fragment growth (Pratchett et al. 2015). Another possible reason for a faster growth in the fore reef habitat in some species would be that they increased their heterotrophic feeding to maintain a positive energy balance (Houlbrèque & Ferrier-Pagès 2009; Bongaerts et al. 2011). Nonetheless, we did not measure flow rates or the contribution of heterotrophic feeding in our study. Additionally, the higher abundance of parrotfishes on the fore reef compared to the sandy lagoon channel insured the grazing of turf and other algae on coral trees, which can lower the competition with corals (Russ 1984; Bonaldo et al. 2014). In fact, the lagoon nursery was highly impacted by floating macroalgae that got tangled in nylon strings and coral branches, mostly *Turbinaria ornata*, *Dictyota* sp. and *Sargassum* sp.. These species are direct competitors of corals, and can significantly reduce the survival and growth of coral fragments (Stiger & Payri 1999; van Woesik et al. 2018; Clements et al. 2018). Even periodic cleaning does not necessarily improve the growth of coral fragments in contact with competitive macroalgae (Lustic et al. 2020).

## Photophysiology

Another possible explanation for a higher growth rate observed in a fore reef site is that photo-acclimation associated with a strong reduction in photosynthetic pigment occurs in very shallow sites (Torres et al. 2007; Richier et al. 2008), followed by a subsequent energetic resource limitation compared to fore reef sites. Photophysiological changes in *A. nasuta* and *A. globiceps* were not investigated, but *A. hyacinthus* was chosen as a proxy for all studied species to better apprehend the mechanisms allowing to maintain a constant growth rate in distinct habitats. First, we documented that symbiont density was strongly reduced in *A. hyacinthus* fragments after one year in the fore reef nursery relative to those maintained at the lagoon nursery. This contrasts with previous works that reported an increase in symbiont

density with depth (Ladrière et al. 2014; Polinski & Voss 2018; Sivaguru et al. 2021), to compensate the lack of light of the deeper environment. Nonetheless, a higher symbiont density with increasing depth is not a universally observed trend. Wild colonies can display a decreased symbiont density at greater depths (McCloskey & Muscatine 1984; Masuda et al. 1993; Fitt et al. 2000), and transplanted fragments can show lower or equal symbiont densities when moved to deeper sites (Dustan 1979; Bongaerts et al. 2011; Cohen & Dubinsky 2015). The assumption behind such changes in symbiont density is that a lower symbiont density could minimize self-shading in sites where light is already limited (McCloskey & Muscatine 1984). Also, our work revealed that for *A. hyacinthus,* symbiont density was strongly positively correlated to the growth rate, with an even more pronounced relationship for fragments living in the fore reef nursery than those in the lagoon nursery. This corroborates with previous studies that found positive links between symbiont density and growth in *Acropora* (Wright et al. 2019; Cornwell et al. 2021).

If symbiont densities are reduced in fore reef fragments and growth is tightly linked to symbiont density, one would expect an inhibited growth in the fore reef nursery compared to the lagoon nursery. However, the growth of *A. hyacinthus* fragments was the same in both nursery habitats. This can be explained by the interaction of symbiont density and the cellular chlorophyll *a* content. Despite fragments from the fore reef nursery having a lower symbiont density, the symbionts harbored a higher cellular chlorophyll *a* content than those from the lagoon nursery, which allowed to compensate for the low number of symbionts. This is consistent with previous studies indicating that cellular chlorophyll *a* in corals increases with the depth gradient (Falkowski & Dubinsky 1981; Dubinsky et al. 1984; Masuda et al. 1993), in low light experiments (Titlyanov et al. 2001; Langlois & Hoogenboom 2014; Fong et al. 2021) and in transplantation experiments (Cohen & Dubinsky 2015; Tamir et al. 2020). Thus, corals that acclimate to low light have higher light harvesting abilities through a higher chlorophyll concentration (Dubinsky et al. 1984). The cellular chlorophyll $c_2$ measured in our experiment had the opposite trend than cellular chlorophyll *a*, and decreased on the fore reef. Similarly, the ratio of chlorophyll $c_2/a$ has been shown to decrease with increasing depth (Lesser et al. 2010; Nir et al. 2011; Polinski & Voss 2018). This might be due to the available light spectrum at deeper sites, which could alter the ratio of photosystems PS I to PS II (Nir et al. 2011), thus relying more on chlorophyll *a*. Also, the ability of corals to transform blue light into orange-red light, which is better absorbed by chlorophyll *a*, through fluorescent protein pigments, could explain the higher prevalence of chlorophyll *a* in deeper corals (Smith 2017). Together, these results confirm that a photo-acclimation took place in less than a year when corals were transplanted from a shallow lagoon habitat to a deeper fore reef habitat, contributing to corals maintaining an equal survival and growth in both habitats.

## Influence of species and size

After symbiont density and chlorophyll content, the strongest predictors for *A. hyacinthus* growth were the species and the size of the fragment. These were also the strongest predictors for the maturity and growth of all five species. For instance, *A. nasuta* and *A. striata* had the

relatively highest growth rate (155.3 and 131.6%/year), followed by *A. hyacinthus* (118.5%/year), *A. retusa* (103.1%/year), while *A. globiceps* had the lowest growth rate (60.8%/year), which corroborates the high variability of growth rates documented for *Acropora* species (Pratchett et al. 2015; Madin et al. 2016). While *A. hyacinthus* is commonly used in coral studies and shows similar growth rates in other nurseries (Morikawa & Palumbi 2019; Howlett et al. 2021), the four other species are barely represented in coral restoration projects. As they swing freely, suspended corals do not have to resist wave and current energy, thus can develop a lighter skeleton than when attached to a substrate (Kuffner et al. 2017), allowing for a faster size increase relative to attached corals (O'Donnell et al. 2017). Also, the five studied species probably have different skeleton accretion strategies, *A. globiceps* forming thicker and stronger branches than others thus showing a slower growth (Kahng et al. 2024), followed by *A. retusa*.

While the size of fragments did not impact their survival chance, it strongly governed the subsequent coral growth, the smaller fragments growing proportionally faster than the larger ones. In fact, fragments measuring less than 50 $cm^2$ increased their surface area more than twice as fast as fragments larger than 100 $cm^2$. Our findings corroborate those concerning other species of *Acropora* (Lirman et al. 2014; Dehnert et al. 2022; Rapuano et al. 2023) and massive species (Yap et al. 1998; Sam et al. 2021): smaller colonies growing proportionally faster than larger colonies. Small coral fragments will thus prioritize clonal growth until they reach a threshold size where they become less vulnerable (Raymundo & Maypa 2004; Doropoulos et al. 2012), to then invest in sexual reproduction and reduce investment in clonal reproduction. Indeed, fragments that had reached 200 $cm^2$ had a significantly higher probability to be mature, but the size to reach maturity strongly depended on the species. *A. hyacinthus*, *A. retusa* and *A. nasuta* fragments matured at smaller sizes and were thus more likely to be mature than *A. striata* and *A. globiceps* fragments. Size and age typically determine the onset of maturity in corals (Hall & Hughes 1996), although asexually propagated fragments can spawn at a much smaller size than the maturity threshold described for wild colonies (Rapuano et al. 2023). However, the timing of fragmentation can strongly impact the reproductive output: if done before or during early vitellogenesis, the reproduction of the fragment can be completely blocked for up to three years (Okubo et al. 2007).

In our work, laboratory spawning was confirmed for fragments of *A. hyacinthus,* that spawned over three months, from October to December, with a peak in November, and *A. nasuta* and *A. retusa* over two months, in October and November. This corroborates previous spawning patterns of these species in Mo'orea (Carroll et al. 2006). Split spawning, over more than one lunar month (Shimoike et al. 1993; Foster et al. 2018), was observed in four out of five species and within 29.7% of colonies of origin , meaning that even if they were genetically identical, almost a third of fragments lost the spawning synchrony with their clone-mates and colony of origin after over a year of being separated. This could be a consequence of the different time of fragmentation between clone-mates, which can alter the reproductive synchrony (Okubo et al. 2007). Also, as shaded areas of a colony can spawn later than areas exposed to light

(Shimoike et al. 1993), perhaps the relative position of each clone-mate on the coral tree allowed some to benefit from more light than others, thus maturing faster. Another study found that the skeletal structure can show significant variability within genetically identical clone-mates four months after fragmentation, potentially because of small scale position effects or the age of individual corallites (Zilberberg & Edmunds 1999). While it is interesting to consider that clonal fragments could gradually grow to become separate entities by altering their physiology and even their reproductive timing, very few studies have tested the differentiation of clone-mates after fragmentation.

## Conclusion and recommendations

Considering our results, both nursery habitats offer their advantages and drawbacks. While the lagoon nursery offered an easier access, a better protection from strong swells and an enhanced probability for corals to be mature, the fore reef nursery necessitated less maintenance because of fewer macroalgae, and showed an improved growth for two out of five species. The fore reef habitat thus seems ideal for an improved outcome of nursery survival and growth, especially since these are the main objectives of the nursery phase in coral restoration. There was, however, a trade-off between the energy invested in reproduction and in growth, with a strong size dependence of the investment. While the smallest fragments grow the fastest, only the largest fragments will reach maturity. The increased growth of small fragments relative to adult colonies can thus be used to maximize coral production. If productivity is the aim of a restoration project, then starting off with fragments less than 50 $cm^2$ will yield the best results, although the onset of maturity will be compromised. The best strategy would be to focus on fast growing species like *A. nasuta* and *A. hyacinthus* (Howlett et al. 2021), which additionally reach maturity at a smaller size thus sooner than other species. Nonetheless, a fast growing rate can be associated with a lower investment in thermal tolerance (Little et al. 2004; Jones & Berkelmans 2010). If the aim is to enhance the future thermal tolerance of coral fragments, then the variable temperature regime of the lagoon might be more beneficial than the stable conditions of the fore reef (Safaie et al. 2018). With the changes in symbiont density, chlorophyll *a* and $c_2$ content, we confirmed that *A. hyacinthus* fragments acclimated to the new environment on the fore reef, which allowed them to maintain a fast growth even at a site with less light availability. Growing corals on the fore reef can thus be considered without too many trade-offs concerning growth and physiology. A change of location to a deeper site might be considered if environmental conditions become unfavorable in the lagoon, due to increasing pollution, turbidity, disease prevalence or algal competition. In the context of the degradation of coral reefs worldwide, optimizing restoration practices might be a way to compensate anthropogenic threats, although the reduction of greenhouse gas emissions should be a priority when aiming to preserve coral reefs.

## Acknowledgements

As guests, we recognize and give thanks for the land and water resources of Mo'orea, and to the traditional owners of the land. We would like to acknowledge all the CRIOBE staff and students who helped during the experimental setup and data collection, especially Yann Lacube, Guillaume Iwankow, Hugo Bischoff, Camille Gache, Colette Buisson, Mathieu Reynaud, Clement Castrec, and Clementine Violette. A special thanks to all the staff and students who helped with macroalgae removal in the lagoon nursery. This research was funded by the AFR Grant from the FNR Luxembourg.

## 5. Supplementary material

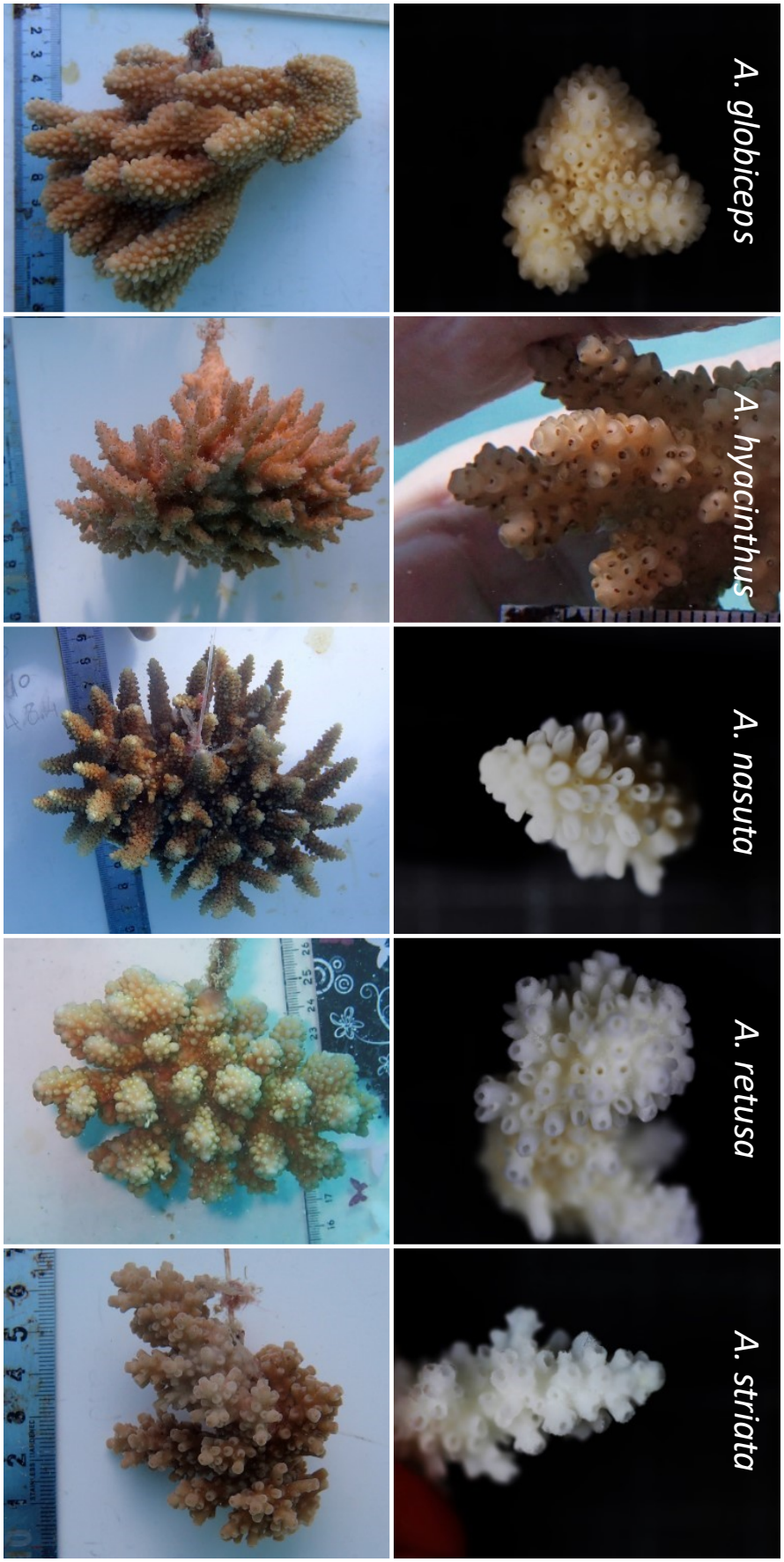



**Figure 2.S1.** Photographs of *Acropora globiceps*, *A. hyacinthus*, *A. nasuta*, *A. retusa* and *A. striata* nursey fragments, with close-ups of bleached skeletons.

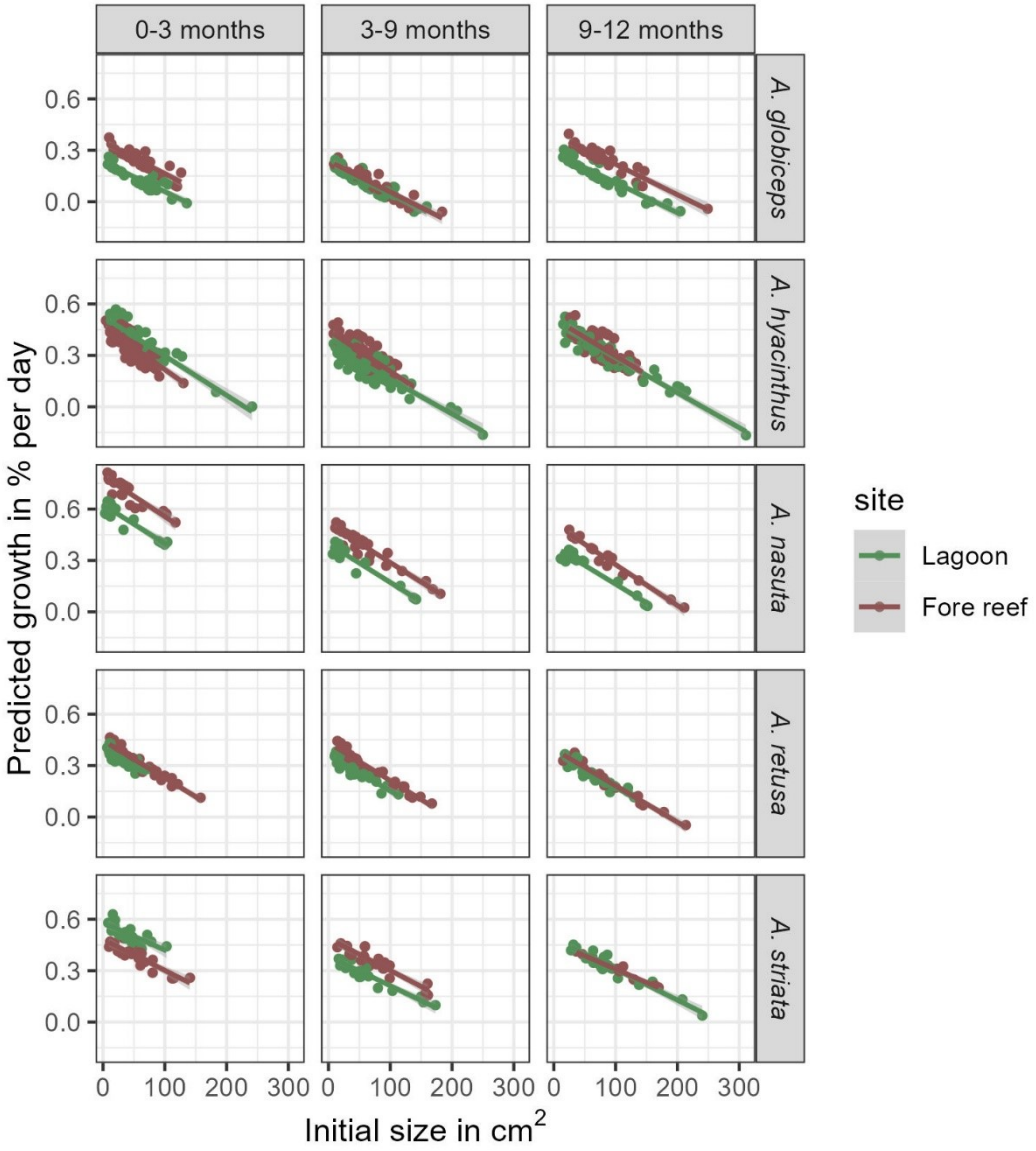



**Figure 2.S2.** Linear mixed model predicted growth of corals in percentage of their initial size per day according to their initial size in $cm^2$, the time of measurement and the species, with a linear regression and a ribbon showing the 95% confidence interval.

# Chapter 3

## Transplantation of nursery-reared *Acropora cytherea* alters growth, survival and symbiont assemblages, independently of varying environmental gradients

# Transplantation of nursery-reared *Acropora cytherea* alters growth, survival and symbiont assemblages, independently of varying environmental gradients

**Running head:** *Acropora cytherea* transplantation

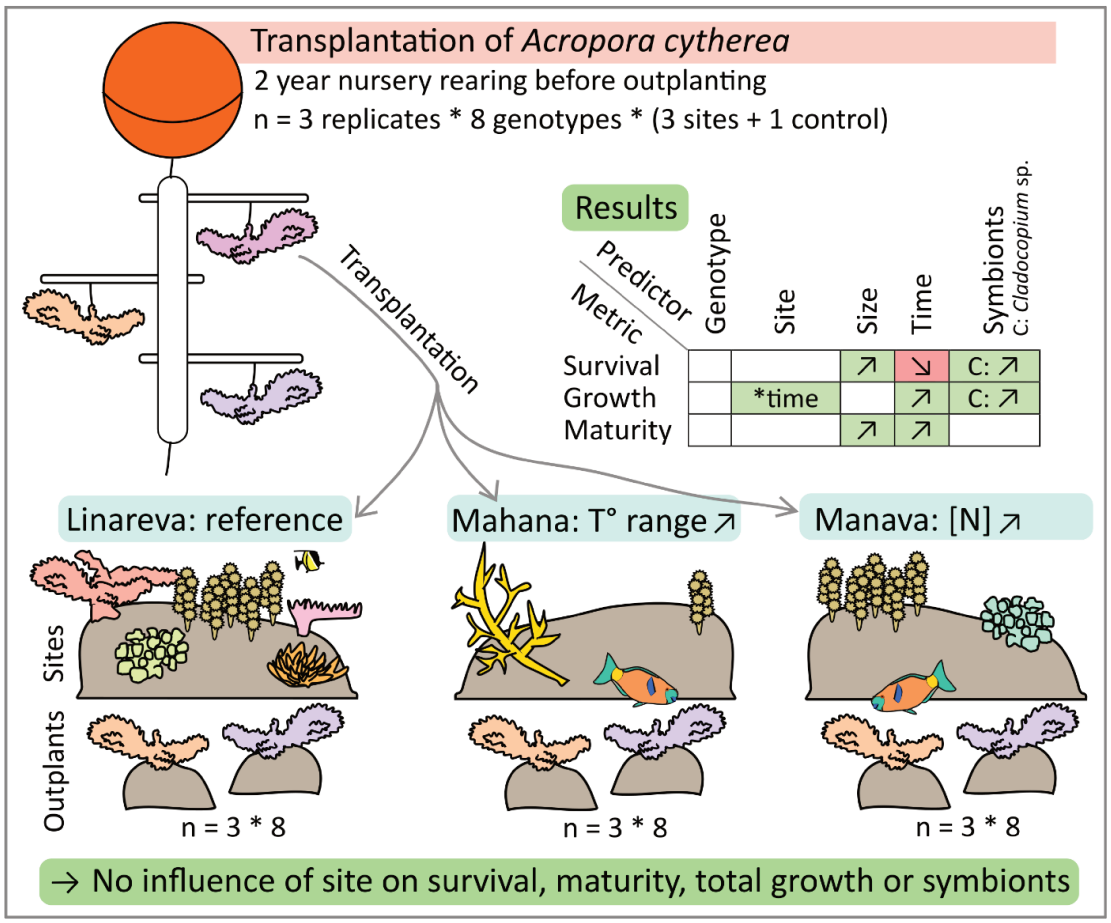
Camille Leonard[1], Caroline Dubé[1,2,3], Julie Stojakovic[1], Nicolas Derome[3], Laetitia Hédouin[1,2]

[1] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, BP 1013, 98729 Papetoai, Mo'orea, French Polynesia

[2] Laboratoire d'Excellence "CORAIL", Papetoai, Mo'orea, French Polynesia

[3] Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec City G1V 0A6, Canada.

## Graphical abstract



## Abstract

Despite coral transplantation being practiced throughout all coral reef regions, the interactions between the environmental conditions of the transplantation site, time, coral genotype, size, and symbiont diversity relative to restoration success remain poorly understood. Long-term monitoring is often lacking, and while survival and growth are frequently reported, other metrics such as maturity or reproductive output are not analyzed. By transplanting nursery-reared *Acropora cytherea* to three lagoon sites with varying environmental conditions (coral cover, temperature, nutrients) we aimed to compare their fitness (survival, growth, sexual maturity) and algal symbiont diversity over a 26-month period. One site was characterized by a healthy wild population of *A. cytherea* and was therefore qualified as the reference site, while the other two were more impacted by human pressures and showed less coral cover. The strongest predictors for survival and maturity were size and time, with larger fragments showing significantly higher chances to survive and to produce gametes. Growth was influenced by an interaction of time and site, with a strong initial decrease in size for corals planted at sites with seeming high predation rates. After the first year, all sites showed a positive growth rate that was similar to the one observed at the nursery site, underlining the need to account for an acclimation time before comparing transplantation success among sites. Symbiont diversity was highly genotype-specific and the abundance of *Cladocopium* was correlated with a higher survival and growth rate, while the opposite trend was observed for *Durusdinium*. As site did not influence the colonies' fitness, coral transplantation can be successful as a restoration approach for degraded sites lacking keystone species.

# 1. Introduction

Climate change is now critically threatening coral reefs worldwide (Hoegh-Guldberg et al. 2018), leading to coral mass mortality during bleaching events after a prolonged heat stress (Coles & Brown 2003; Hédouin et al. 2020; Yadav et al. 2023). In addition, other local anthropogenic pressures threaten the health of coral reefs, including urban expansion, eutrophication, coastal constructions, pollution and overfishing (Nakano 1990; Edinger et al. 1998; Hughes et al. 2013). In the light of these pressing threats, coral restoration has become a commonly used approach to increase coral cover at degraded sites and/or enhance reef resilience through the selection of certain advantageous traits. Restoration techniques are highly diversified, ranging from local coral gardening (Rinkevich 2000) to large-scale larval seeding projects (Doropoulos et al. 2019a). New approaches are also being tested to increase coral resistance to future disturbances through assisted evolution, such as acclimatization through epigenetic mechanisms, selective breeding or the manipulation of the coral microbiome and algal symbionts (Van Oppen et al. 2015; Drury et al. 2022; Quigley et al. 2023). Currently, the most common restoration method is coral transplantation after a nursery phase, where asexually propagated fragments are grown in nurseries with favorable conditions, then outplanted to degraded reefs (Bowden-Kerby 1997; Rinkevich 2000). Outplanting corals can benefit the reef by increasing coral cover as well as fish biomass (Dehnert et al. 2023), and recruitment rates can be higher at transplanted sites compared to healthy ones, underlining the potential of new outplants in attracting coral larvae (Montoya-Maya et al. 2016), although this likely depends on the chosen site (Ferse et al. 2013). Transplantation can also increase reef resilience by introducing missing species or new genotypes with enhanced performances, such as heat or disease resistance.

Despite increasing coral resilience, transplantation can negatively affect survival and growth of fragments. Initial survival and growth of outplanted corals can be substantially lower compared to undisturbed wild colonies or to nursery corals (Lirman et al. 2014). This is likely due to changes in energy allocation towards healing (Omori 2019) or to predation stress (Clark & Edwards 1995). As such, growth and survival are the most frequently reported fitness metrics in transplantation studies, but the different methodologies used to estimate growth rates can introduce significant discrepancies between metrics (Boström Einarsson et al. 2020a). Such methodological differences in growth data collection and analysis make comparisons of restoration outcomes difficult. Despite studies highlighting that size is significantly altering the survival of coral fragments, most published data in coral gardening did not consider the size (Boström-Einarsson et al. 2020a), which limits our capacities to accurately interpret the metrics and making informed decisions in coral reef management. Moreover, this body of work focuses mainly on certain species with *A. cervicornis*, *P. damicornis* and *S. pistillata* being the most represented species in transplantation studies (Boström-Einarsson et al. 2020b, 2020a). More results concerning less represented species, as well as a more standardized monitoring, would help understand differences in transplantation outcomes.

The survival of coral fragments is also influenced by the environmental conditions at the transplantation site. Depending on the levels of nutrient concentration, nutrient enrichment has been reported to increase the biomass, algal symbiont density and photosynthetic performance in *Pocillopora* fragments (Becker et al. 2021). However, nutrient enrichment can increase the prevalence of coral bleaching and mortality (Burkepile et al. 2020), disturb the balance in nutrient exchange between symbionts and corals, reduce light availability by enhancing phytoplankton density, and cause the proliferation of macroalgae (Dubinsky & Stambler 1996), and is generally linked to massive decline in coral reef ecosystems (Bellwood et al. 2004). Other factors such as particulate organic carbon, photosynthetically active radiation and temperature anomalies have been shown to influence the survival of coral outplants (Foo & Asner 2021), while transplant fitness can be higher at sites with higher water flow, salinity, and pH fluctuations (Barott et al. 2021).

Studies have also shown that corals of the same species can respond differently to changing environmental conditions depending on their genotype (Maneval et al. 2021). The origin of coral fragments can also influence their innate resistance to environmental changes. For instance, outplants from highly variable environments can have a better survival rate compared to outplants from less variable environments (Bay & Palumbi 2017). Specific associations with algal symbionts can also allow coral fragments to grow faster or better resist environmental stress (Jones & Berkelmans 2010; Morris et al. 2019). While coral symbiont communities are often stable over time (Thornhill et al. 2006; Rouzé et al. 2019), changes can occur when transplanted to a drastically different environment (Gantt et al. 2023), during cold-water events (LaJeunesse et al. 2010) or heat waves (Cunning et al. 2018). This can result in detrimental effects on corals' survival and growth (Howells et al. 2012; Gantt et al. 2023). Other studies showed no variation in symbiont communities despite reciprocal transplantations to sites with varying thermal regimes, turbidity or acidity (Hauff et al. 2016; Haydon et al. 2021). These varying results concerning the flexibility or stability of symbiont communities could be due to different coral species, genotypes or environmental conditions (Dilworth et al. 2021).

Establishing new genetically diverse populations in areas showing a decline of wild population through transplantation of several genotypes could significantly contribute to species recovery once the new colonies become mature (Baums et al. 2005; Vollmer & Palumbi 2007). The reproductive capacity of outplanted colonies can be a good indicator of reef replenishment, but this fitness trait is often lacking from transplant monitoring results due to their short duration (Hein et al. 2017). Only few transplantation studies have reported reproductive data, showing that the reproduction was stopped for most corals one to three years following fragmentation and transplantation (Young et al. 2012; Carne et al. 2015). This was even accentuated for smaller fragments (Smith & Hughes 1999) or those that were fragmented during early vitellogenesis (Okubo et al. 2007, 2009). Nonetheless, for long-term studies monitoring reproductive capacities five years after transplantation (Diraviya Raj et al. 2015; Martínez-Castillo et al. 2023), results showed that spawning timing and maturity of outplants

can then become similar to that of wild colonies. In addition, nursery-bred outplants can retain a substantially higher larval production than wild colonies under the same environmental conditions, suggesting that the benefit of nursery-rearing can be maintained even years after transplantation (Horoszowski-Fridman et al. 2020). This underlines the potential of coral transplantation in increasing reef recovery rates, and the importance to monitor the reproductive output of outplants for a sufficiently long duration to assess restoration success.

Before investing in large-scale restoration programs, preliminary studies need to be undertaken. Because the influence of environmental conditions on transplantation outcomes are still poorly understood, and long-term surveys remain scarce, more experiments are needed to evaluate the long-term impact of transplantation on coral fitness. The present experiment is a case study on *Acropora cytherea*, a large tabular species that is essential for creating habitat and surface complexity in the lagoons of French Polynesia. However, this keystone species often disappears in degraded sites. Despite its demise, *A. cytherea* is not often considered in global restoration studies, i.e. mentioned in less than 1% of transplantation or nursery studies (Boström-Einarsson et al. 2020b, 2020a). After two years of nursery rearing, our aim was to compare the transplantation outcomes for a 27-month period at three sites selected based on their contrasting levels of degradation: one site is characterized by a healthy *A. cytherea* population and qualified as the reference site, the second by a moderate coral and algal cover, and the third by a low coral and high algal cover. We expect the survival, growth, and maturity to be higher at the reference site, while reduced at the moderate and highly impacted sites. Considering the strong expected differences between sites, we hypothesize that genotype effect would be weaker than site effect. We also expect symbiont diversity to influence the survival and growth of coral colonies and symbiont assemblages to change with time and site as corals acclimate to their transplantation sites. Similar to other studies, we hypothesize that mature colonies would be less abundant among transplanted colonies than in undisturbed nursery corals.

# 2. Methods

## 2.1 Transplantation of *Acropora cytherea* from the nursery

The present study was undertaken in the lagoon of Mo'orea, French Polynesia. The coral nursery was located in the channel slope between the Hilton hotel and Paopao pass, from five to seven meters depth (17°29'01.1"S 149°50'03.9"W). It was made of floating "coral trees" that are composed of hollow 2 m PVC tubes, with a 50 mm diameter. They were anchored in the sand with underground anchors (Gripple) and floated vertically with 10 mm nylon boat line and an orange round rigid plastic mooring buoy. The buoy stayed at least 3 m below the sea surface so as not to disturb boat traffic. The trees were drilled twice on 10 levels, and two 40 cm fiberglass branches (10 mm diameter) were inserted and attached at each level. Each branch had four holes, one to secure a cable-tie around the tree trunk, and three to support suspended coral colonies. Being situated in the channel, the lagoon nursery was subjected to abundant floating macroalgae (*Turbinaria ornata*, *Dictyota* sp. and *Sargassum* sp.) that got caught in the suspended coral colony. Consequently, a bimonthly clean-up was organized, where scuba-divers removed the macroalgae with gloves, dish brushes, or knives. Small (5-10 cm) *A. cytherea* fragments were collected from adult colonies from two sites in the lagoon in 2018 (Linareva 17°33'13.4"S 149°53'08.5"W and Vaiare 17°31'06.9"S 149°46'18.8"W).

After growing coral fragments to large colonies for more than two years, the 12 biggest colonies from eight genotypes were selected for the transplantation experiment (n = 96). On February 2 2021, all corals were sampled using scuba-diving (detailed in 'Coral sampling and DNA extraction' paragraph). In the following days, three colonies from each of the eight genotypes were planted at 1 m depth at Mahana (17°29'13.6"S 149°53'15.6"W) and Manava (17°28'36.7"S 149°48'21.4"W) on the north coast, and Linareva (17°33'13.4"S 149°53'08.5"W) on the west coast (Fig. 3.1). Sites were chosen for their varying coral diversity and anthropogenic influences. Manava, assumed to be the most impacted site, is close to a populated area of the island and a hotel on stilts. Mahana was further away from human constructions, but very close to a channel with dense boat traffic. Linareva on the west coast is more isolated and relatively undisturbed (Rouzé et al. 2019), and was chosen as the reference site. Of the chosen sites, only Linareva harbored healthy and abundant wild *A. cytherea* populations, while Mahana exhibit only few wild colonies and Manava none.

Colonies were planted onto bare bommies with a paste of 5 kg prompt marine cement for 1 kg super Sikalite. Three colonies from each eight genotypes were kept in the nursery as controls. The eight chosen genotypes were confirmed to be separate genotypes through successful cross-fertilization during previous experiments (Leonard et al. 2024a). One week after transplantation, colonies at Mahana and Manava were already paler, smaller and had many bite marks (see Fig. 3.S6 for representative photos).

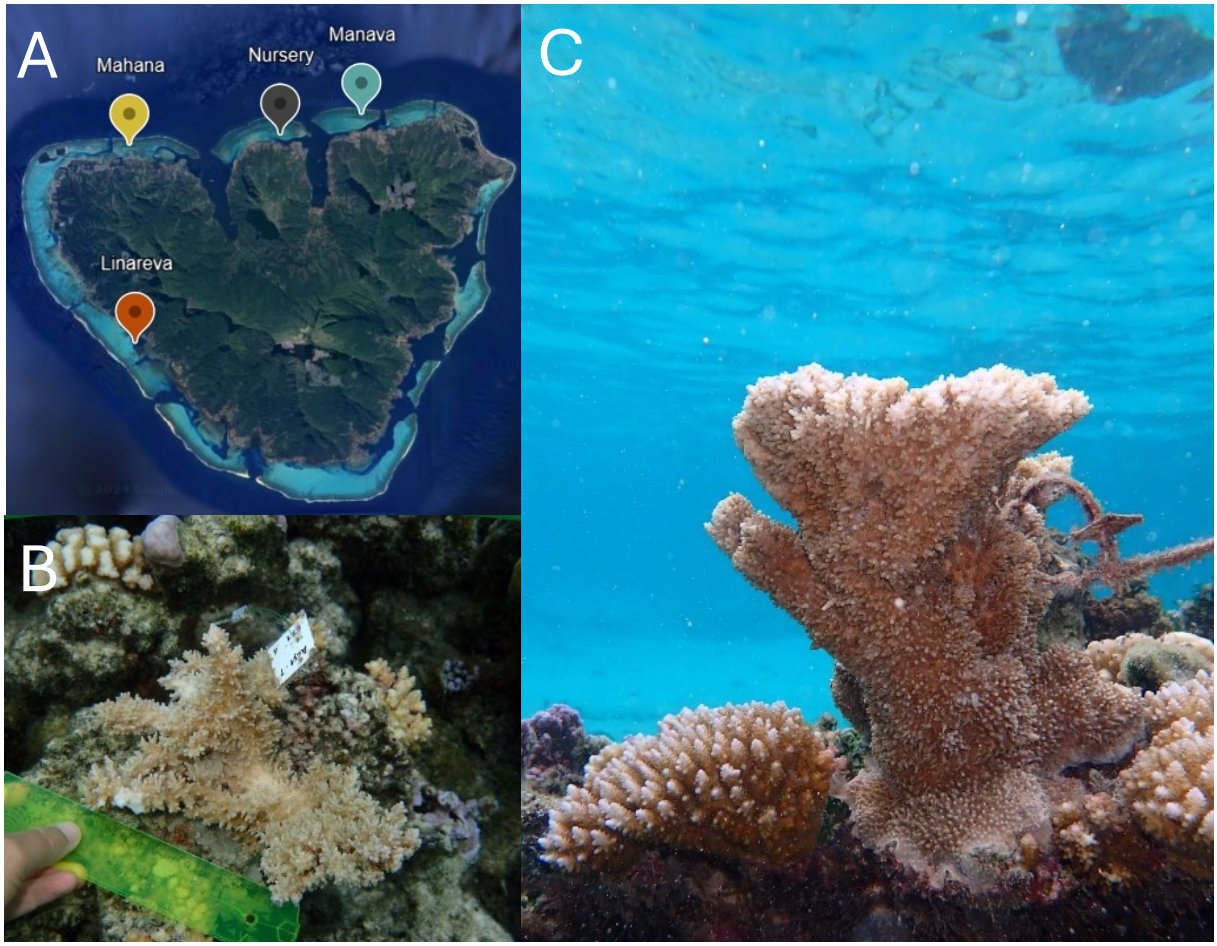



**Figure 3.1.** Map of transplantation and nursery sites around the island of Mo'orea, French Polynesia (A). Photo of *A. cytherea* colony at transplantation (B) and the same colony two years after transplantation (C).

## 2.2 Environmental conditions

Temperature loggers (HOBO Water Temperature Pro v. 2 Data) were deployed at the four sites for 12 months, but due to material failure, only three consecutive months could be overlapped in all four sites.

Three sand and water samples from each site were analyzed to estimate the concentration of nitrogen dioxide ($NO_2$), nitrate ($NO_3$), ammonium ($NH_4$), phosphate ($PO_4$) and orthosilicic acid ($Si(OH)_4$) with a Technicon AutoAnalyzerII. Nutrient data was also gathered from Mo'orea Coral Reef LTER data (Moorea Coral Reef LTER & Carpenter 2023). The nitrogen content percentage of *Turbinaria* sp. was measured in six sites around the lagoon, with sites LTER 1, 2, 5 and 6 corresponding roughly to the locations of our nursery site, Manava, Linareva and Mahana, respectively (Moorea Coral Reef LTER & Carpenter 2023). *Turbinaria* nitrogen content is considered be a better indicator of long-term nutrient concentration compared to a single

water sample (Donovan et al. 2020). Data from 2020 were used, only from the backreef sampling (Moorea Coral Reef LTER & Carpenter 2023).

To characterize the biotic environment, underwater visual surveys were carried out at the three transplantation sites. Surveys were performed in June 2022. 25-meter long transects (n = 6 per site) were deployed randomly at 1-2 m depth. Percent cover of the benthos was estimated using the Line Intercept Transect method (Canfield 1941). Benthic organisms were visually noted into four main categories: 1/ corals, 2/ algae further divided into *Dictyota* sp., *Halimeda* sp., turf, *Turbinaria* sp. and other algae), 3/ substrate classified as dead corals (rubble or massive *Porites* sp.), 4/ sediments and 5/other organisms (giant clam, sponge and soft coral). Corals were identified to the genus level.

## 2.3 Coral fitness

Survival, partial mortality, partial or total bleaching was recorded every month in the first year at each site, and every four months thereafter. Photos were taken top down with an Olympus TG3, with a ruler parallel to the camera, next to the base of each coral. The fisheye distortion was corrected on GIMP (GIMP Development Team 2019) (Main distortion = 37, edge distortion = 2). The size of the fragment was measured using ImageJ (Schneider et al. 2012). The maximum diameter and the largest surface area in $cm^2$ (with the freehand polygon selection tool, around the outline of the largest coral surface) were measured. Photos were taken immediately after transplantation, a week after, then every month for the first year and every four months thereafter.

Relative growth was expressed similarly to previous studies (Neal et al. 2015; Suggett et al. 2019; Howlett et al. 2021), i.e. the percentage of increase per day, calculated as the surface area difference (final-initial) divided by the initial surface area and the number of days between two measures. The initial surface area of our *A. cytherea* colonies ranged from 19.1 to 460.3 $cm^2$, averaging 167.3 $cm^2$. The maturity of the fragments was recorded after transplantation two weeks before the full moon of September 20 2021 and at the full moon of October 9 2022. A 3 cm branch was broken off from each colony and the inside of the polyps was visually checked. Colonies were noted as either empty, with white eggs or with mature eggs, if these were pigmented pink/reddish.

## 2.4 Coral sampling and DNA extraction

Each coral colony was sampled before transplantation, after 12 months and 27 months (corresponding to the end of the warm season). Small fragments (3-4 cm) were taken with pliers, put into small, labelled zip lock bags, cut at both corners to keep the seawater flowing through. After the dive, the bags were shaken to empty the seawater, then placed in a cooler filled with ice cubes. Back at the research station (fifteen minutes later), they were frozen at –

80°C until further analysis. The following day, 2-10 mm pieces were cut from the frozen fragment and stored at -20°C in a sterile 2 mL tube filled with 96° ethanol.

Coral fragments were washed with PBS 1X, pH 7.4, then placed in a bead beating tube containing 0.13 g of garnet flakes (0.56 - 0.7 mm, from Lysing Matrix A, MP Biomedicals), 95 µL ultra-pure water and 95 µL of Solid tissue buffer (Zymo Quick-DNA™ kit), and one Zirconium-ceramic bead (6.35 mm, from Lysing Matrix A, MP Biomedicals) was added on top. Samples were broken using a high-speed homogenizer FastPrep-24 5 G Instrument (MP Biomedicals, Santa Ana, CA, USA) under the following conditions: speed: 6.0 m/s, time: 30 s, pause time: 60 s, cycles: 3. They were then centrifuged one minute at 15000 g (SL 8R, Thermo Scientific). DNA was extracted from 212 corals using a Zymo Quick-DNA™ kit, following the manufacturer's standard protocol for animal tissues with 2-hours incubation at 55°C for sample lysis using 10 µL of Proteinase K. DNA was quantified with Nanodrop 2000c (Thermo Scientific) and Qubit 4 fluorometer (invitrogen).

## 2.5 Symbiodiniaceae ITS2 library preparation, sequencing, and SymPortal analysis

The Symbiodiniaceae ITS2 region of the rDNA was amplified with a first PCR using the primers SYM_VAR_5.8S2 and SYM_VAR_REV (Hume et al. 2018) with added sequencing adapters (forward: 5' – ACACTCTTTCCCTACACGACGCTCTTCCGATCTGAATTGCAGAACTCCGTGA ACC – 3'; reverse: 5'–GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGGTTCWCTTGTYT GACTTCATGC –3'; Illumina overhang adaptor sequences are underlined) and using the enzyme Q5 High Fidelity DNA Polymerase with the manufacturer's standard protocol (New England, Biolabs). Initial denaturation was at 98°C for 2 minutes, denaturation was at 98°C for 10 seconds, then annealing was at 60°C for 30 seconds, followed by elongation at 72°C for 30 seconds, and the final elongation at 72°C for 10 minutes. After 35 cycles, electrophoresis on 2% agarose gels was done to verify the successful amplification of the ITS2 region. Samples were then purified with AMPure beads [cat #A63880, Beckman Coulter, Pasadena (CA), USA] and quantified by Nanodrop. A second PCR was performed to barcode samples with two indexes. For this PCR, the final elongation was at 72°C for 10 minutes, and 12 cycles were programmed. As for the first PCR, 2% agarose gels were used, followed by purification. Finally, barcoded samples were pooled, and the smallest concentration of DNA was used to equilibrate the quantity of DNA for each sample in the pool. The sequencing was made on Illumina MiSeq in paired-end mode (2 × 300 bp) at the IBIS Genomics Platform (Université Laval, Québec, QC, Canada). Determined sequencing data for this project are available under NCBI BioProject X. Paired-end sequencing reads were submitted to SymPortal for quality control and analysis at SymPortal.org (Hume et al. 2019). Briefly, sequence quality control was conducted as part of the SymPortal pipeline using mothur v1.39.5 (Schloss et al. 2009), the BLAST+ suite of executables (Camacho et al. 2009), and minimum entropy decomposition (MED) (Eren et al. 2015). ITS2 type profiles, representative of putative Symbiodiniaceae genotypes, were

predicted by searching for co-occurring sets of sequences and characterized by specific sets of defining intragenomic ITS2 sequence variants (DIVs). Where ITS2 sequence abundances are reported in this study, we refer to post-MED, rather than pre-MED, abundances.

## 2.6 Cost estimation

The cost estimation is an approximate calculation of the paid work hours (around 8.64 USD/h, mean between intern and technician salaries), fuel consumption of car, boats and dive compressor (price of diesel in 2020 in Polynesia 1.08 USD/L of diesel), purchase of the cement and Sikalite. It comprises the collection of wild fragments from two different sites, the production of around 160 small fragments, their 2-year rearing in an in situ nursery that requires a bimonthly algal removal, then the ouplanting of 72 colonies to three different sites. The initial purchase of large equipment and the value of the work put in for monitoring or data analysis were not taken into account.

## 2.7 Statistical analysis

Data were tested for their normal distribution with the *shapiro.test* function and the homogeneity of variance with the *bartlett.test* function. Significant differences were tested with the *anova_test* function followed by a *pairwise.t.test*, or the *kruskal.test* function followed by the *pairwise.wilcox.test* function when data were non-parametric.

Differences in mean temperatures and nitrogen contents were tested with the *anova_test* function, while differences in temperature maximums, minimums and ranges were tested with the *pairwise.wilcox.test*. Differences in sand and water nutrient concentrations were tested with a Kruskal-Wallis test and pairwise comparisons were done with the *dunn.test* function (Dinno 2024) with a Holm adjustment. Differences between transects were tested with the *adonis2* and *pairwise.adonis* from the *vegan* and *pairwiseAdonis* packages (Oksanen et al. 2009; Martinez Arbizu 2020). Differences in coral and macroalgae cover were tested with the *pairwise.wilcox.test and pairwise.t.test*, respectively.

Differences in survival probability (every four months) were tested with an *Anova* from the *car* package (Bates et al. 2007), on a binomial generalized mixed model, with the *glmer* function from the *lme4* package (Bates et al. 2015), with size, genotype, site, time, state and growth rate as explanatory variables, and colony as a random factor (the measures were linked to the same colony through time). Differences in monthly survival were also tested on a coxph proportional hazards regression model, from the *survival* package (Therneau 2024), with the sites and genotypes as explanatory variables, and added to Table 3.S1 in the supplementary materials. Growth was expressed as a daily increase in surface (percentage of the initial surface), and differences were tested with an *Anova* on an *lmer*, with genotype, site, size, and time as explanatory variables, and colony as a random factor. Correlations between growth and initial size, and between growth, survival and symbiont genera were tested with the

Pearson's test, *cor.test* function. Differences in the probability of maturity were tested with an *Anova* on a binomial *GLMER*, with genotype, site, size and year as explanatory variables, and colony as a random factor. The interactions were left out of this model as they proved to be non-significant. Post-hoc pairwise differences were tested with the *emmeans* function from the *emmeans* package (Lenth et al. 2024). The influence of the site of origin of fragments on survival and growth was tested in initial models, but was non-significant thus removed from the final models.

Changes in Symbiodiniaceae community composition was analyzed with a Permanova on a Bray Curtis distance matrix with 9999 permutations, with the *vegdist*, *adonis2* and *pairwise.adonis* functions (Benjamini-Hochberg adjusted p-value) from the *vegan* and *pairwiseAdonis* packages (Oksanen et al. 2009; Martinez Arbizu 2020), with genotype, time and site as explanatory variables. To account for the low number of replicates within each genotype and each site, transplant sites were grouped as "Transplant" treatment, and the influence of treatment (transplant vs control) on the symbiont composition of each genotype was tested for the 12 months sampling. To graphically represent symbiont communities, the 17 most abundant symbiont strains were kept (percentage > 1%) and the rest were pooled into the different genus (*Symbiodinium*, A; *Cladocopium*, C; *Durusdinium*, D): "Other A", "Other C" and "Other D". For graphical representation of the symbiont profiles, only the 18 most abundant profiles were kept (total abundance > 1%). Dissimilarities between samples were visualized using a principal coordinate analysis (PCoA) using the 'ape' R package.

All data and code used for this study are available on GitHub (https://github.com/CamiLeonard/Cytherea).

# 3. Results

## 3.1 Environmental conditions

### Temperature

The mean daily temperatures between all four sites were not statistically different ($p = 0.554$, Table 3.S1). However, daily maximums, minimums and ranges of temperatures were statistically different between all sites (Table 3.S1 and Fig. 3.S1A). Mahana had significantly higher daily maximums than Linareva and Manava. The nursery, despite being deeper, also showed higher maximums than Manava. Mahana also had lower minimums than Manava and the nursery. All sites had significantly different daily ranges, with Mahana having the highest (1.37 ± 0.05°C) (mean ± SEM), followed by the nursery (1.12 ± 0.04°C), Linareva (1.02 ± 0.04°C), and Manava (0.89 ± 0.03°C). All sites showed a stationary variance throughout the three monitored months (Augmented Dickey-Fuller test, pval = 0.01).

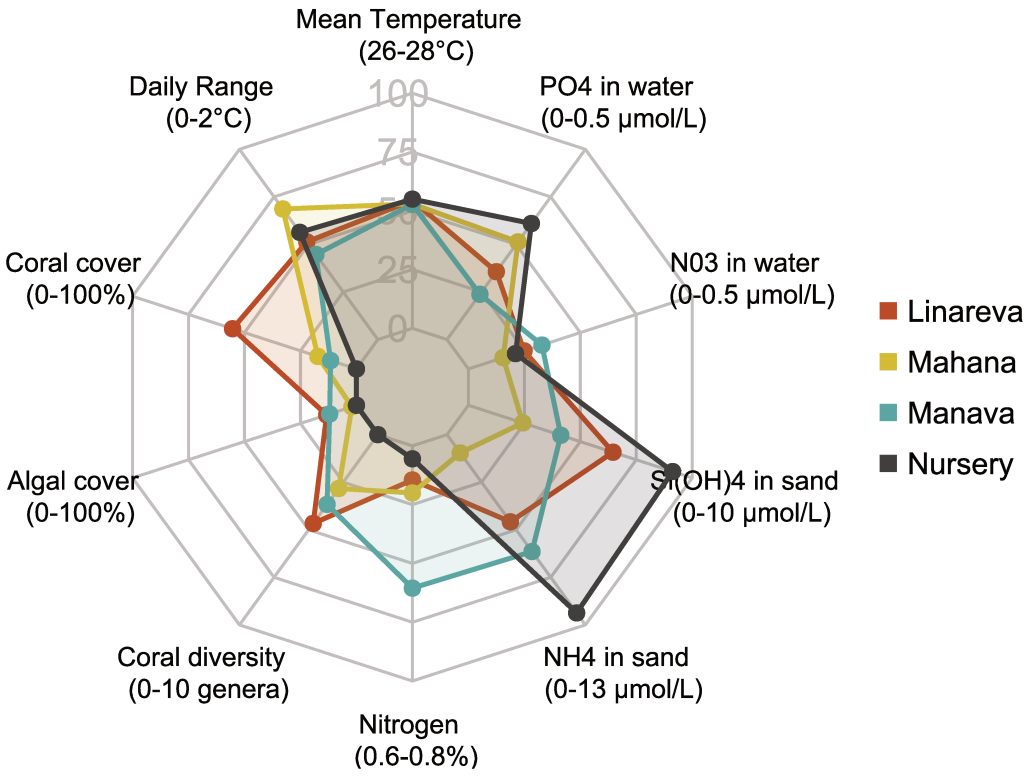



**Figure 3.2.** Comparison of environmental characteristics of three different lagoon transplantation sites and the coral nursery as a control. Lagoon sites are situated at 1 m depth on the North and West coast of Mo'orea, and the nursery is between 6-7 m depth on the North coast. Displayed are mean daily temperatures (no significant differences), nitrogen content in *Turbinaria* sp. (Manava > other sites), surrounding coral diversity (transplantation sites > nursery), surrounding algal cover (Linareva & Manava > Mahana), surrounding live coral cover (Linareva > Mahana & Manava), daily temperature range (daily maximum - minimum) (Mahana > Nursery > Linareva > Manava), $NH_4$ in sand (Nursery > Mahana), $Si(OH)_4$ in sand (Nursery > Mahana), $NO_3$ in water column (Manava > Mahana), $PO_4$ in water (Nursery > Manava).

### Nutrients

Nutrient concentrations varied significantly among all sites, and more specifically for $NO_2$, $NO_3$, $NH_4$ and $Si(OH)_4$ in the sand, and $NO_3$ and $PO_4$ in the water column (KW < 0.05) (Fig . 3.S1B). The nursery had higher concentrations of $NH_4$ and $Si(OH)_4$ in the sand than Mahana (dunn padj < 0.025), Manava had a higher $NO_3$ concentration in the water than Mahana but a lower $PO_4$ concentration than the nursery. According to the Mo'orea Coral Reef LTER data (Moorea Coral Reef LTER & Carpenter 2023), the 2020 N content in *Turbinaria* sp. (g per g of algae) was highest at Manava (0.72 ± 0.02%), and significantly higher than around the nursery (0.61 ± 0.02%), Linareva (0.63 ± 0.03%) and LTER 6 (as an approximation for Mahana) (0.64 ± 0.02%) (Pairwise t test $p < 0.05$).

### Ecological composition

The reference site Linareva differed significantly from the other two transplantation sites based on the benthic composition (Permanova $p < 0.05$, Table 3.S1 and Fig. 3.S2A). Linareva had a significantly higher live coral cover (55.33 ± 4.28%) than Mahana (17.20 ± 3.40%) and Manava (11.53 ± 1.19%) (Fig. 3.2). Mahana had significantly less algal cover (2.03 ± 1.18%) than both Manava (11.97 ± 2.70%) and Linareva (13.30 ± 2.26%). The three transplantation sites did not differ in the overall coral generic richness (3.72 ± 0.32 coral genera per 25 m transect), but each site showed a significantly different generic composition (Permanova $p < 0.05$, Table 3.S1 and Fig. 3.S2B). For instance, Linareva showed a higher percentage of *Pavona* (16.81 ± 5.90% of live coral cover) than other sites, and Mahana a higher percentage of *Porites* (81.53 ± 6.51%) than Linareva (Fig. 3.S2B). Linareva presented overall 3.13 ± 1.22% live *A. cytherea* cover, while Mahana and Manava displayed 0% *A. cytherea*.

## 3.2 Survival

Survival of corals showed a decrease with monitoring time (Fig. 3.3) and was mostly determined by the size of the transplanted fragments (measured every four months) (Table 3.1, Fig. 3.S4A). A 100 $cm^2$ colony showed approximately 80% chance to survive, and a 200 $cm^2$ colony around 95% (Fig. 3.S4A). The mean initial size at transplantation was 174.07 ± 10.47 $cm^2$. The time also had a significant influence on the survival probability (Table 3.1). While no mortality was observed in the first month, survival probability was significantly higher in months 1-4 compared to months 16-20, 20-24, and 24-26 (Fig. 3.3).

The monthly survival probability, without taking size or time into account, was significantly influenced by the interaction of transplantation site and genotype ($p = 0.043$, Table 3.S1). Genotypes 2 and 3 showed lower survival in Manava than in Linareva or Mahana (Fig. 3.S3). For all genotypes combined, no site had a better survival rate than another (Fig. 3.3). Overall survival chance was 68.1% one year after transplantation, and 37.5% after two years. No significant differences were observed between survival on transplantation sites and in the

nursery (Fig. 3.3, Table 3.S1). Also, in the nursery alone, survival did not depend on the colony size.

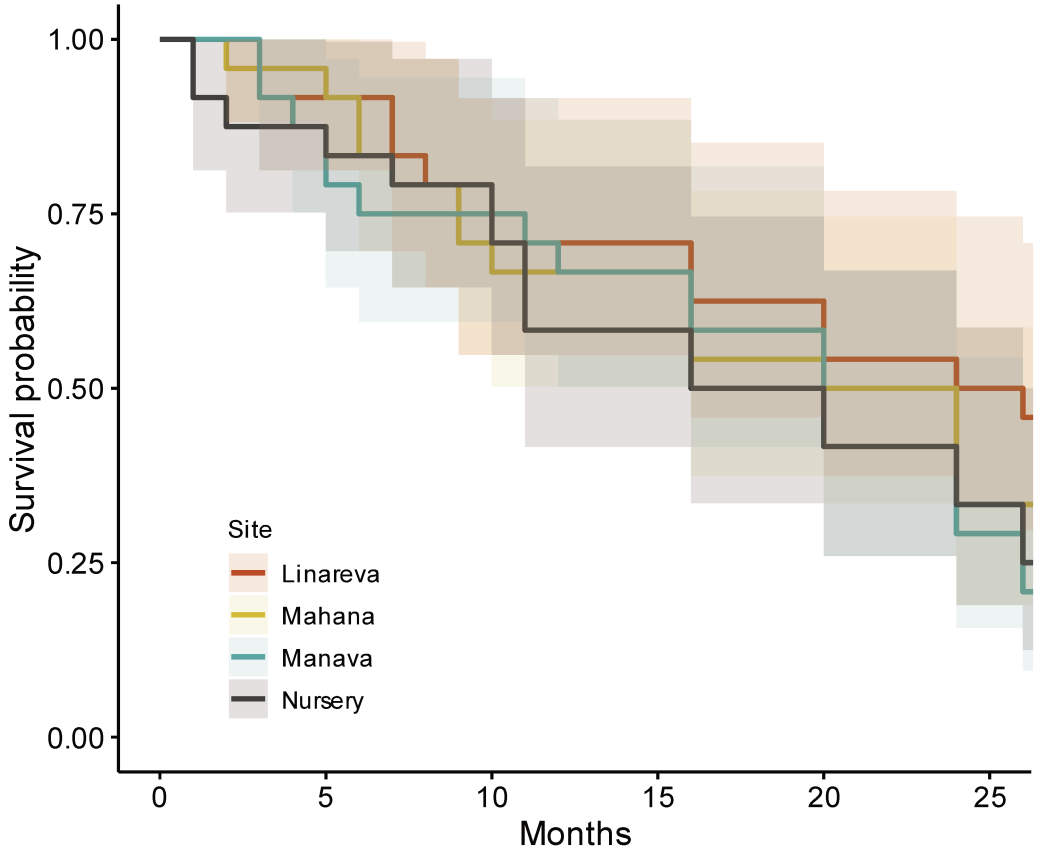



**Figure 3.3.** Survival probability of *Acropora cytherea* colonies transplanted to different lagoon sites or kept in the suspended coral nursery. The line represents the mean and ribbon the 95% confidence interval.

**Table 3.1.** Anova results of predictors best influencing survival, growth, maturity, and symbiont diversity. Bold values indicate statistically significant predictors.

| Response variable | Test | Predictor | $X^2$ (or other) | df | p |
|---|---|---|---|---|---|
| Survival probability | Anova(binomial glm) | **Size** | **24.06** | **1** | **< 0.001** |
| | | Genotype | 8.78 | 7 | 0.269 |
| | | Transplant site | 0.611 | 2 | 0.737 |
| | | **Time** | **16.13** | **7** | **0.024** |
| | | State | 3.69 | 5 | 0.595 |
| | | Growth | 0.000 | 1 | 0.984 |
| | | Genotype*Site | 17.77 | 14 | 0.218 |
| Growth (Healthy outplants) | Anova(lmer) | Initial size | 0.27 | 1 | 0.606 |
| | | Genotype | 2.43 | 7 | 0.932 |
| | | Transplant site | 0.84 | 2 | 0.658 |
| | | **Time** | **32.34** | **7** | **< 0.001** |
| | | Genotype*Site | 19.48 | 14 | 0.147 |
| | | Genotype*Time | 49.37 | 48 | 0.418 |
| | | **Site*Time** | **28.32** | **14** | **0.013** |
| | | Genotype*Site*Time | 73.09 | 57 | 0.074 |
| Maturity | Anova(binomial glmer) | Genotype | 5.02 | 7 | 0.658 |
| | | Site | 2.68 | 3 | 0.262 |
| | | **Size** | **6.06** | **1** | **0.014** |
| | | **Year** | **4.83** | **1** | **0.028** |
| Symbiont diversity | Permanova (Bray Curtis) | **Genotype** | **147** | **7** | **< 0.001** |
| | | **Site** | **49.19** | **3** | **< 0.001** |
| | | **Time** | **24.87** | **2** | **< 0.001** |
| | | **Genotype*Site** | **8.56** | **20** | **< 0.001** |
| | | **Genotype*Time** | **5.81** | **13** | **< 0.001** |
| | | Site*Time | 1.71 | 3 | 0.130 |
| | | **Gen*Site*Time** | **2.19** | **11** | **0.003** |

## 3.3 Growth

The growth of transplanted corals was mostly influenced by their visual state, i.e. healthy, full, or partial bleaching, and partial mortality or predation (Table 3.S1). Predation was inferred when size shrank, or obvious bite marks were observed (see Fig. 3.S6 for representative photos). Obvious signs of predation were noted in 11% of colonies in Mahana (monitored every 4 months), 14% in Manava and 0% in Linareva. Partial mortality was due to spontaneous bleaching or algal competition and was noted in 20.3% of colonies in Mahana, 28.4% in Manava and 26.3% in Linareva.

When analyzing healthy and impaired corals separately, the time was the factor influencing coral growth the most, in interaction with the transplant site (Table 3.1 & 3.S1). The initial size and genotype did not influence coral growth. A negative growth was often observed due to partial mortality caused by predation (see Fig. 3.S6 for representative photos of the impact of predation), algal competition or partial bleaching.

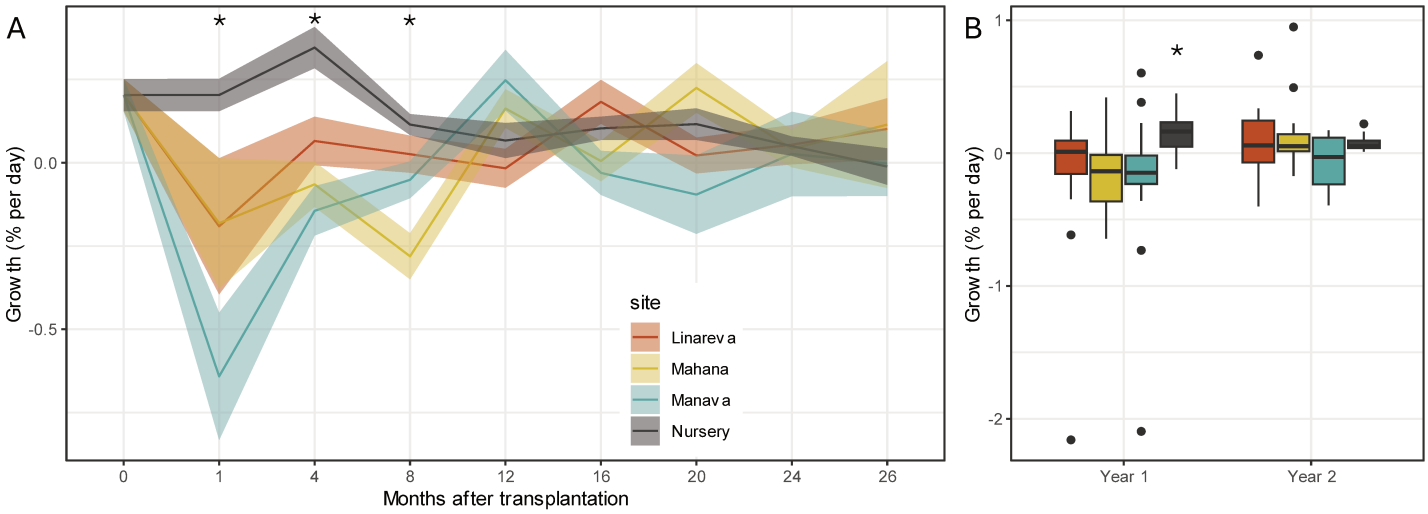



**Figure 3.4.** Growth of *A. cytherea* colonies, expressed by the percentage of initial size increase per day, between four sites (three transplantation sites and one control nursery site). A) The line represents the mean and ribbon the standard error of the mean. Stars at one month after transplantation represent significant differences between the nursery and Manava, at four months between the nursery and Manava and Mahana, at 8 months between Mahana and the nursery and Linareva. B) The line on the boxplot represents the median, the box the median 50%. The stars represent the significant differences between the nursery and all transplantation sites at $p < 0.05$.

In the first four months, growth among all outplants was significantly lower at Manava than in Linareva (Fig. 3.4A, Table 3.S1). Between four to eight months after transplantation, Linareva and Manava colonies grew faster than Mahana colonies. Other than these punctual differences, transplantation sites did not influence growth rates. Overall, growth of transplanted colonies was slowed down in the first year after transplantation, as shown by a significantly higher growth for the corals at the nursery in the first year (Fig. 3.4B), but increased in the second year after transplantation (-0.14 ± 0.05% per day in the first year after transplantation, 0.05 ± 0.04% in the second year). Among only healthy colonies, growth was on average 0.20 ± 0.03% per day in the first year and 0.16 ± 0.02% in the second year. No differences in growth were found for healthy colonies between transplantation sites, except after 8 months where Linareva still had an increased growth relative to Mahana.

When compared to suspended control corals in the nursery, growth was significantly lower at Manava in the first month and after four months (Fig. 3.4A, Table 3.S1). Four and eight months after transplantation, nursery colonies grew faster than at Mahana. After a year, there were no significant differences in the growth rates between treatments (transplant vs control). Overall, nursery growth rates were significantly higher than those at all transplantation sites (Fig. 3.4B), but these differences disappeared in the second year. Similarly to healthy transplanted colonies, nursery colonies tended to grow less than half as fast in the second year relative to the first year (0.16 ± 0.03% in the first year, 0.07 ± 0.02% in the second year). In the

nursery, growth was negatively correlated with the initial size, but this trend was not observed for all the transplantation sites (Table 3.S1).

## 3.4 Maturity

The probability to be mature was predominantly explained by the size of the colony, but also by the interaction of genotype, site and year ($p < 0.05$, Table 3.S1). In September 2021, seven months after transplantation, 15 ± 8.2% of the colonies were mature in Linareva and 17.39 ± 8.08% in the nursery, while no colonies were mature in Mahana or Manava. In 2022, there were significantly more mature colonies (48.78 ± 7.9%) than in 2021 (8.97 ± 3.26%). Twenty months after transplantation (October 2022), colony maturity was influenced by size and genotype, but no differences were found among sites (Fig. 3.5A). Post-hoc tests revealed no statistical differences between genotypes. On transplantation sites, immature colonies were significantly smaller (145.32 ± 15.07 $cm^2$ in 2021, 165.71 ± 27.18 $cm^2$ in 2022) than mature ones (235.47 ± 25.8 $cm^2$ in 2021, 421.56 ± 47.29 $cm^2$ in 2022). However, nursery colonies did not vary in size according to their maturity (Table 3.S1, Fig. 3.5B).

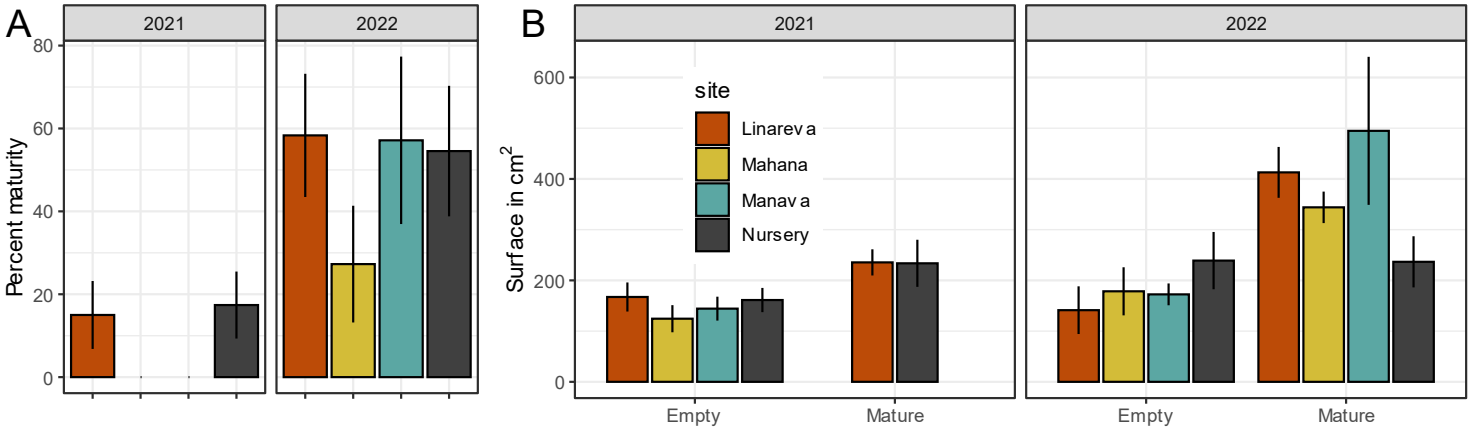



**Figure 3.5.** Probability of transplanted and control (nursery) colonies to be mature seven and 20 months after transplantation (A). Size of *A. cytherea* colonies according to their maturity seven and 20 months after transplantation (B). Error bars represent the standard error of the mean.

## 3.5 Symbiont assemblages

From the 212 coral fragments, a total of 201 unique Symbiodiniaceae sequences (with a mean of 29.2 ± 0.8 per sample) were retrieved from the sequencing of the ITS2 amplicons that belonged to three genera: *Symbiodinium* (n = 78), *Cladocopium* (n = 70), and *Durusdinium* (n = 53) (corresponding to previously described clades A, C, and D, respectively) (LaJeunesse et al. 2018). While no sequences were present in all samples, the majority were from the genus *Cladocopium* (52.0%, mostly C3ae, C3bj and C3p), followed by *Durusdinium* (37.3%, mostly D1, D1u and D4), and *Symbiodinium* (10.7%, mostly A1 and A1ee). A total of 29 distinct ITS2

profiles were identified by SymPortal analysis, with a mean of 2.1 ± 0.06 profiles per sample (Fig. 3.S8).

Overall, genotypes, time and sites, all interacted to significantly influence symbiont community composition (Table 3.1). For each site separately, differences in symbiont assemblages were explained by the interaction of time and genotype. At T0 and T27, only genotype had a significant influence on the symbiont composition.

Before transplantation, at T0 in the nursery, each genotype had its own unique symbiont community (Pairwise Permanova $p < 0.05$, Table 3.S3, Fig. 3.6). Genotypes 3 and 7 were characterized by a dominance of *Cladocopium* (98.2% and 63.6% respectively, with C3ae and C3bj being the dominant sequences in both), while genotypes 5, 8, 9 and 10 were dominated by *Durusdinium* (87.1%, 99.4%, 96.6%, 89.0% respectively, with D1 as the dominant sequence) (Fig. 3.6). Genotypes 2 and 4 were dominated by two genera, *Symbiodinium*-*Cladocopium* for G2 (53.3% and 46.6%, respectively, with A1 and C3ae as dominant sequences) and *Cladocopium*-*Durusdinium* for G4 (60.8% and 39.1%, respectively, with C3ae and D1 as dominant sequences) (Fig. 3.6).

Twelve months after transplantation (T12), symbiont assemblages were significantly influenced by the interaction of site and genotype (Permanova $p < 0.001$, Table 3.S2). For each genotype individually, no differences were found among sites. However, when comparing the nursery to all outplanted colonies after 12 months, significant changes in symbiont composition were found for genotype 8 and 10 after transplantation (Pairwise permanova, $p < 0.05$, Table S2). For instance, once outplanted, genotype 10 showed a significantly decreased abundance of *Durusdinium* D1 (Kruskal test KW $p = 0.04$) relative to its nursery counterpart, but also increase its abundance in *Symbiodinium* A1ee (KW $p = 0.04$) and *Cladocopium* C3ae (KW $p = 0.04$) (Fig. 3.6). A similar trend was observed for genotype 8 where the abundance of *Durusdinium* decreased in favor of *Cladocopium* in outplants relative to the colonies remaining in the nursery (Fig. 3.6).

After 27 months, differences were also found among genotypes (Table 3.S2, $p = 0.018$).

Symbiont communities changed significantly over time in the nursery, but in interaction with the genotype (Permanova $p < 0.001$, Table 3.S2). When looking at each genotype individually, only genotype 2, 4 and 8 significantly changed their symbiont community over time in the nursery (Permanova $p < 0.05$, Table 3.S2). T0 was significantly different from T12 and T27, but T12 and T27 were not different from each other.

Over time and independently of site, genotype 2 significantly changed its symbiont community between T0 and T12 and between T0 and T27, mostly by losing its *Symbiodinium* symbionts (A1, pairwise Wilcoxon test pW $p = 0.004$) in favor of *Cladocopium* (mostly C3ae, KW $p = 0.001$). Genotype 3 changed between T0 and T12 and between T0 and T27 (pairwise Permanova $p = 0.003$), by losing both *Symbiodinium* and *Durusdinium*. Genotype 4 changed only between T0 and T12 by losing its *Durusdinium* (D1, KW $p < 0.001$) in favor of *Cladocopium* (mostly C3ae, KW $p < 0.001$). Genotype 5 significantly changed at each monitoring time, for instance by

gaining *Symbiodinium* (A1, pW p = 0.008) and *Cladocopium* (C3ae, pW p < 0.05) after 12 months. Genotype 7 changed between T0 and T12 and between T0 and T27, by gaining *Cladocopium* (C3ae, KW p = 0.02) and losing *Durusdinium* (D1 pW p < 0.004). Genotype 8 changed only between T0 and T12, by losing *Durusdinium* (D1, pW p < 0.001) and gaining *Cladocopium* (C3ae pW p < 0.001). Genotype 9 and 10 both showed a significant interaction between site and time. However, post-hoc pairwise permanovas failed to pick up significant changes in symbiont community within any site or any time. Overall, genotype 9 changed between all timepoints, by losing *Durusdinium* (D1, pW p < 0.05) and gaining *Cladocopium* (C3ae pW p < 0.05). Genotype 10 also significantly changed between all timepoints, by gaining *Symbiodinium* (A1ee, pW p < 0.05) and losing *Durusdinium* (D1, pW p < 0.05). Thus, there was an overall decrease in *Durusdinium* abundance for a strong dominance of *Cladocopium* (83.9%, see fig. 3.S9) after 27 months, and a relatively stable *Symbiodinium* abundance.

By analyzing the links between symbiont genera, survival, growth and maturity, some significant correlations were found (Fig. 3.7 & 3.S5). *Cladocopium*, found mostly in genotypes 2, 3, 4 and 7, was positively correlated to colony growth ($r$ = 0.26) and survival ($r$ = 0.19), while *Durusdinium*, found in majority in genotypes 5, 8, 9 and 10, was negatively correlated to survival ($r$ = -0.21) and growth ($r$ = -0.22) (Fig 3.7 & 3.S5). *Symbiodinium* showed no significant correlations. Size and growth were positively correlated to lifespan ($r$ = 0.29 and 0.63) and negatively to mortality ($r$ = -0.42 and -0.53), while maturity was positively correlated to lifespan ($r$ = 0.41) and size ($r$ = 0.57) (Fig. 3.7 & 3.S5). The correlations were observed both with all sites combined and when omitting the nursery site.

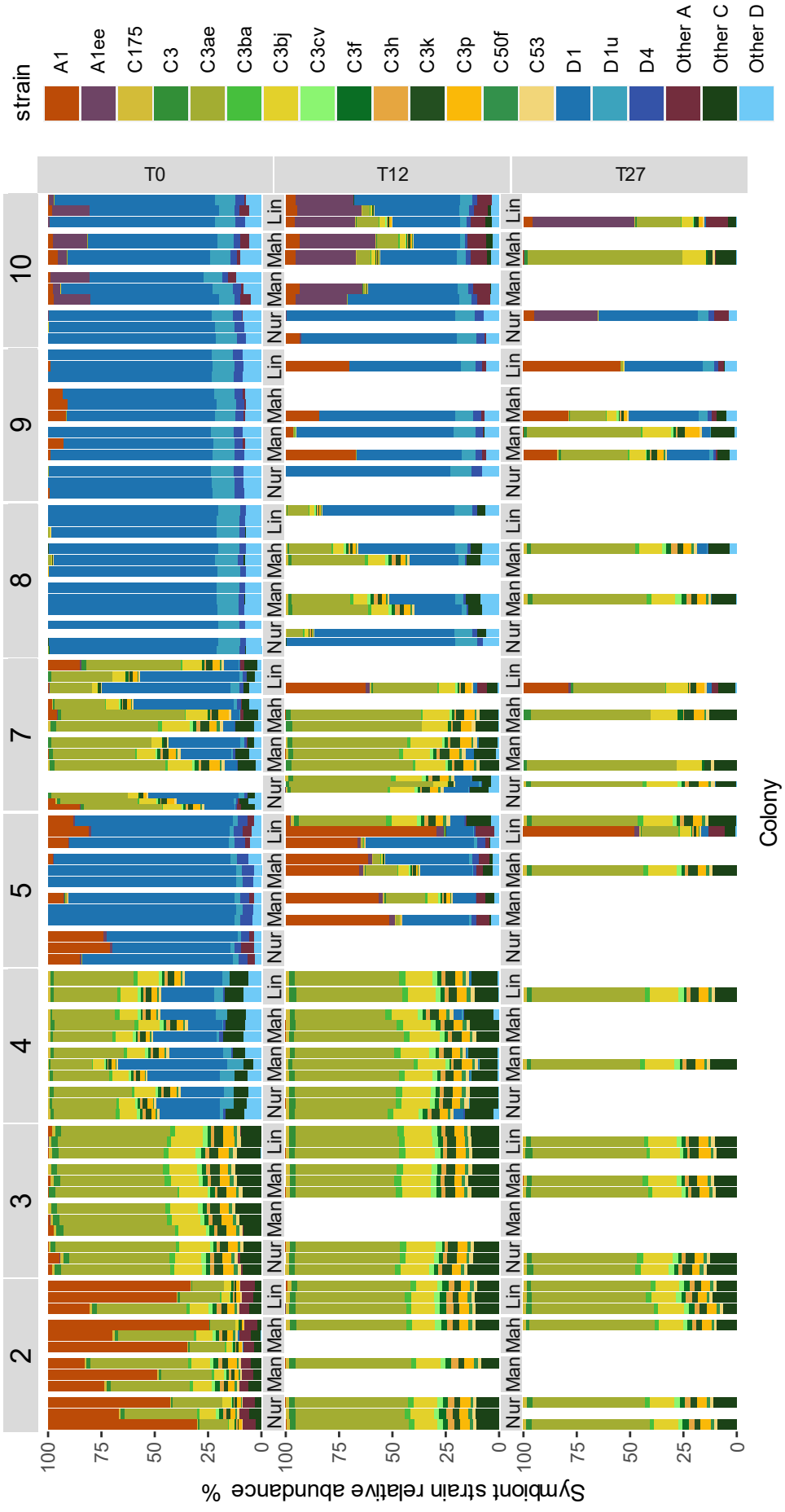

strain
A1
A1ee
C175
C3
C3ae
C3ba
C3bj
C3cv
C3f
C3h
C3k
C3p
C50f
C53
D1
D1u
D4
Other A
Other C
Other D
T0
T12
T27
Colony
2
3
4
5
7
8
9
10
Nur
Man
Mah
Lin
Symbiont strain relative abundance %
100
75
50
25
0

**Figure 3.6.** Symbiont diversity of eight *Acropora cytherea* genotypes (2-10), in the nursery (Nur) before transplantation (T0), then 12 and 27 months after transplantation (T12 and T27) to different lagoon sites (Man = Manava, Mah = Mahana, Lin = Linareva). Symbiont strains were identified through Symportal and summarized by keeping the 17 most abundant strains and the remaining were grouped by clade. Proportions of each strain belonging to the genera *Symbiodinium* sp. (sequences A in shades of red), *Cladocopium* sp. (sequences C in shades of green and yellow) and *Durusdinium* sp. (sequences D in shades of blue) are shown. A missing bar represents a mortality event, except in the nursery as different colonies from the same genotype (7 and 8) were chosen to replace dead ones.

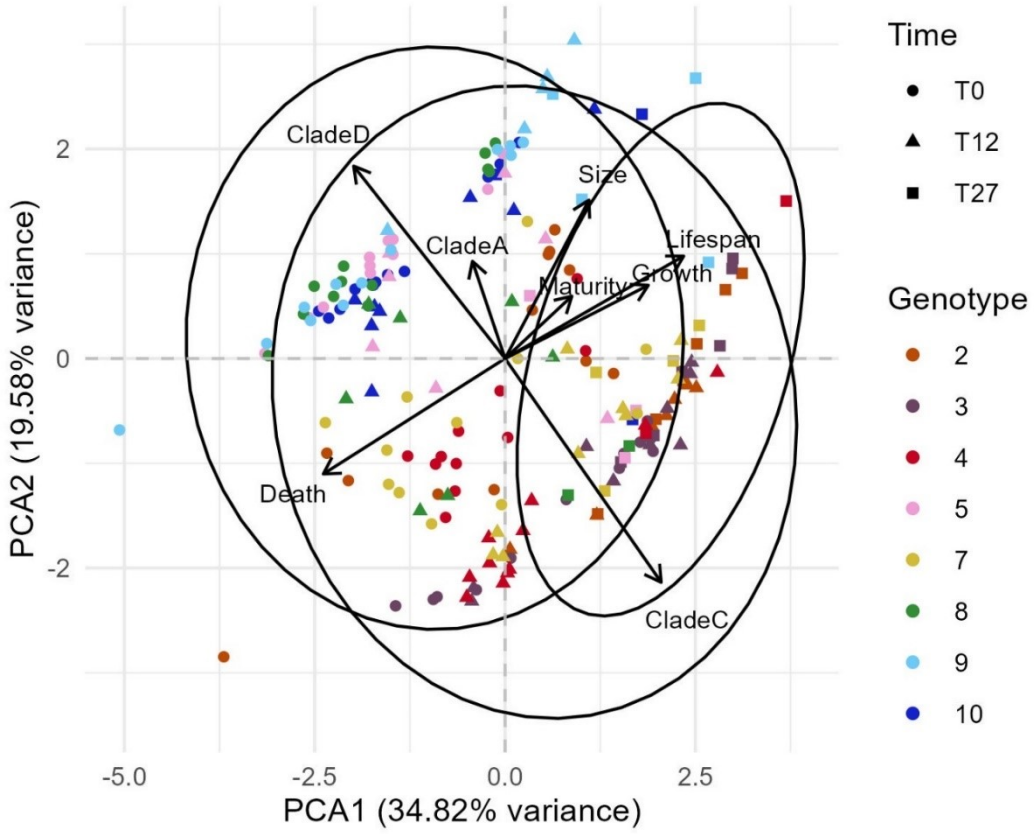



**Figure. 3.7.** PCA of transplanted *A. cytherea* death probability, lifespan, growth rate, size, maturity and symbiont diversity depending on genotype and time (before transplantation, after 12 and 27 months).

## 3.6 Production cost

Without taking into account the purchase of dive equipment, the regular monitoring or any data analysis, the price of producing around 160 fragments, growing them out for two years in an in situ nursery that requires a bimonthly algal removal, then outplanting 72 of them to three different sites, required an investment of approximately 3057 USD. This amounts to around 62 USD for each of the 49 surviving colonies after one year, and 113 USD for a two-year-old transplant (for 27 survivors). The most expensive aspect of the project was the maintenance of the nursery over the two years (total of 2387 USD).

## 4. Discussion

To develop effective conservation strategies, it is imperative to apprehend how coral transplants respond to different environments. By studying fitness traits of coral fragments in contrasting environments through a transplantation experiment, we can identify the most resistant and resilient coral genotypes. The coral *Acropora cytherea* was used to assess our capacity to restore different sites in the local context of French Polynesia. French Polynesia is subject to low anthropogenic disturbances but has suffered repetitive crown-of-thorn invasions, cyclones and bleaching events (Adjeroud et al. 2018; Pérez-Rosales et al. 2021a). Our goal was to provide information for targeting restoration efforts towards individuals that are most likely to survive and thrive in different environments. To do so, we compared the survival, growth, maturity, and symbiont communities of transplanted *A. cytherea* colonies in three contrasting sites around the lagoon of Mo'orea. The reference site (Linareva) had a higher coral cover and lower algal nitrogen, while the most impacted site (Manava) had low coral cover, high macroalgal cover and high algal nitrogen and nitrate content. The intermediate site (Mahana) was also characterized by the highest daily temperature range, low nutrient levels, low coral and algal covers. Despite these environmental differences, growth, survival, maturity, and symbiont communities were not influenced by the transplantation site. Symbiont communities were mostly influenced by host genotype, survival and maturity by the colony size, and growth by time. These results do not support our original hypothesis that sites with different environmental conditions strongly impact the transplantation outcomes.

Overall, the survival of transplanted colonies was 68.1% after one year and 37.5% after two years. Previous studies have revealed a high variability of the survival of *Acropora* corals after transplantation, ranging from 32% to 95% after one year and around 35% after two years (Garrison & Ward 2012; Young et al. 2012; Omori & Nakamura 2016), which is similar to our results. The outplants and nursery colonies had similar mortality rates, suggesting that the transplantation had no deleterious effects on survival even though corals that were kept in the nursery are presumably less subjected to predation or high irradiance. The survival of transplanted colonies depended mostly on their size, with the larger ones showing a significantly higher survival as previously observed in various transplantation experiments (Smith & Hughes 1999; Okubo et al. 2007; Forrester et al. 2014). For example, a 100 $cm^2$ colony showed approximately 80% chance of survival, while a 200 $cm^2$ colony around 95%, which can be due to the increased vulnerability of small fragments to fish predation (Koval et al. 2020). Another possible explanation for the low survival of seemingly small fragments is that partial bleaching was often observed a few months before colony death and was considered as a shrinking size.

We also noticed a decrease in survival of transplanted coral fragments with time, with lower survival in the last ten months relative to the first four months. The mortality of outplanted corals may be due to challenging new conditions and/or failure of attachment methods (as in Ferse 2010; Forrester et al. 2014). In our case, the first period of transplantation showed little

mortality due to very low attachment failures, but mortality increased as time progressed. Such mortality is likely due to the transplanted corals facing persistent environmental stressors such as heat waves and increasing algal competition (van Woesik et al. 2018; Donovan et al. 2020; Adam et al. 2021). Unfortunately, no long-term environmental data were available to correlate to the long-term survival of our coral outplants. Similar to previous observations, mortality rates can increase two or three years after transplantation (Ferse 2008), underlining the importance of a long monitoring.

Average growth was -0.14% per day in the first year and 0.05% per day in the second year. Both Manava and Mahana (i.e. the most and intermediate impacted sites) showed punctual reduced growth rates relative to the reference site Linareva, which could be explained by an enhanced initial predation pressure. In previous studies, the growth of outplants has also been found to be limited by the predation from crown-of-thorn starfish, parrot-, butterfly- and triggerfish (Neudecker 1979; Cabaitan et al. 2015; Shaver et al. 2017), but also acclimation processes to new environments or thermal regimes (Howells et al. 2013). Algal competition (by *Dictyota*, *Sargassum* and *Turbinaria*) has also been observed to cause partial mortality thus growth reduction (as in van Woesik et al. 2018). The growth rates in the nursery were higher than on transplantation sites in the first year. As such, the stress caused by the transplantation itself (Lirman et al. 2010; Forrester et al. 2014) can induce a reallocation of resources towards healing, attachment to the substrate (Omori 2019), and/or a change in morphology (Kuffner et al. 2017). In fact, suspended nursery corals can have a lighter skeleton than colonies attached to the substratum, as they do not have to withstand strong currents (Kuffner et al. 2017). This can explain why suspended nursery fragments showed a faster growth than attached fragments initially (as in O'Donnell et al. 2017). Right after transplantation, corals likely had to put their resources towards increasing their skeletal density (Kuffner et al. 2017). The limited growth of our outplants observed during the first year might therefore be linked to a combination of algal competition, bleaching, predation, environmental stress and changes in skeletal density.

In the second year, after an acclimation phase, the surviving colonies showed positive growth rates, irrespective of the transplantation site and similar to nursery growth rates. A similar pattern has been found for *A. cytherea* for which the growth was slow up to seven months after transplantation, with some corals shrinking or losing tissues, but showed an increased growth after this period (Clark & Edwards 1995). For transplanted colonies, the initial size did not influence the growth, although we hypothesized that small fragments would grow faster than larger ones proportionally to their initial size. Because most outplants recorded as small were colonies that experienced partial mortality, this might induce an impaired growth due to the dead branches that are still attached to the living colony. The growth of nursery colonies, which did not suffer partial mortality, was influenced by their initial size with small ones growing faster than large ones, proportionally to their initial size. This trend was also observed in other nursery-reared corals (Lirman et al. 2014; Dehnert et al. 2022; Rapuano et al. 2023). This can explain why the growth at the nursery was initially higher, then decreased as the

colonies grew larger. By the end of the experiment, nursery colonies were smaller than outplants, which suggest that they can only reach a certain size because of the physical space between each colony on a nursery branch. Thus, outplanting seems beneficial for corals to grow in an unrestricted space.

Once corals reach a certain size, they usually become sexually mature (Hall & Hughes 1996). Thus, when outplanted colonies reach maturity, they can contribute to the local larval recruitment (Doropoulos et al. 2019a) and mix their genetic material with that of wild populations, which underlines the importance to monitor this fitness trait. Here, the maturity of outplants was not different among the transplantation sites, but was mostly influenced by the time and size of the colony, with larger ones having more chances to become mature. In the second year, thus 20 months after transplantation, 49% of the colonies were mature, with a mean size of 421 $cm^2$, relative to only 9% after seven months following the transplantation, with a mean size of 235 $cm^2$.

As maturity probability was similar in transplantation sites and the nursery, nothing indicates that there has been egg resorption after transplantation as we first hypothesized. In previous transplantation studies, the act of fragmentation was suggested to cause egg resorption and a regression to an immature state for up to three years (Okubo et al. 2007; Smith & Hughes 1999; Young et al. 2012; Carne et al. 2015). This could explain why only 9% of the colonies spawned in the nursery or the transplantation sites two years after their initial fragmentation. Although the maturity of the colonies in the nursery was not influenced by their size, this trait was the strongest predictor of maturity for the transplanted corals. Since corals usually show a size-dependent onset of maturity (Hall & Hughes 1996; Randall et al. 2020), we can assume that corals in the nursery grow to a specific size before running out of space and invest in reproduction rather than lateral expansion even at a smaller size (Martínez-Castillo et al. 2023).

Although coral genotype has been shown to influence the growth and survival of outplants in previous studies (Rinkevich 2000; Ladd et al. 2017; O'Donnell et al. 2017; Maneval et al. 2021), this was not the case in our study. Nevertheless, differences among genotype fitness could also be linked to the origin of the colony further influencing the survival, with coral growth being higher at the home site compared to the transplantation sites (Forrester et al. 2013). In our case, fragment origin was not linked to survival or growth rates. While coral genotype did not influence the fitness traits of transplanted corals, we found that symbiont composition of outplants was strongly determined by genotype, as each of the eight genotypes used for the transplantation had its unique symbiont community, and this for each monitoring time. Symbiodiniaceae assemblages are known to be highly genotype-specific, irrespective of host habitat (Yamashita et al. 2014; Quigley et al. 2016, 2017; Dilworth et al. 2021; Dubé et al. 2023). For instance, symbiont communities of *P. acuta* showed no significant changes in composition after nine months of transplantation to warmer and more acidic conditions (Haydon et al. 2021). Rouzé et al. also observed genotype-specific symbiont associations over

18 months in *A. cytherea* across varying environmental gradients (Rouzé et al. 2019), but also observed sporadic and reversible switching events (Rouzé et al. 2016).

Although genotype was the strongest predictor for changes in symbiont community composition, the interaction of genotypes, time, and site was another determinant factor. Seven out of eight genotypes altered their symbiont community composition during the transplantation experiment. These temporal changes occurred independently of the site or transplantation treatment for five genotypes, meaning that nursery colonies experienced similar shifts in symbiont assemblages through time. Genotypes 8 and 10, both strongly dominated by *Durusdinium*, altered their symbiont diversity after 12 months by increasing their proportion of *Cladocopium* and *Symbiodinium*, respectively, and this for all transplantation sites. The other two genotypes (5 and 9) dominated by *Durusdinium* also gradually shifted towards a dominance of *Symbiodinium* and *Cladocopium*. As these are more generalist genera, they might confer advantages such as an increased growth rate to their hosts in the absence of heat waves (Cunning et al. 2015). Similarly to our findings, ex situ nursery-raised juveniles can display a strong initial dominance of *Durusdinium,* but shift towards a prevalence of *Cladocopium* once outplanted to natural sites (Jandang et al. 2024). The dominance of *Durusdinium* was suggested to be caused by elevated temperatures in the rearing tanks (Jandang et al. 2024).

Some of these symbiont genera were found to be significantly correlated with survival and growth. Although no significant correlations have been found between *Symbiodinium* and fitness traits, a higher abundance of *Cladocopium* species was associated with an increased survival, life expectancy and growth, while a prevalence of *Durusdinium* showed the opposite trend. In a previous study on *A. cervicornis*, *Symbiodinium* and *Cladocopium* did not generate differences in the growth rates of transplanted corals (Lirman et al. 2014). Others have revealed that associations with *Cladocopium* benefit coral growth, while *Durusdinium* is generally associated with a higher thermal tolerance but a reduced growth at ambient temperature (Jones & Berkelmans 2010; Cunning et al. 2015; Palacio-Castro et al. 2023). A dominance of *Durusdinium* might have reduced the growth rate of the outplants, potentially resulting in lower survival rates. *Durusdinium* can also be associated with an impaired reproductive output compared to *Cladocopium* (Jones & Berkelmans 2011), but no correlations with the maturity were found. A gradual shift from a dominance of *Durusdinium* towards a dominance of a more generalist *Cladocopium* might thus coincide with the absence of acute thermal stress observed during the monitoring period. Symbiont shuffling occurs very slowly in the absence of bleaching (Baker 2001). While thermal stress was high during the summer of 2019 (Speare et al. 2022) and still relatively high in 2020 in Mo'orea (Edmunds et al. 2024), lower temperatures were recorded during our transplantation experiment between 2021-2023 (Fig. 3.S10). In this context, outplants could have benefitted from an increased growth and survival through the uptake of *Cladocopium*.

Despite the potential to increase the resilience of degraded populations, the transplantation of adult corals is labor-intensive and expensive. Based on the hours of labor, fuel consumption

and the outplants' survival rate, a quick estimation of the cost producing a one-year colony would be 62 USD, or 113 USD for a two-year-old colony. This is similar to the 88 USD necessary to produce 20-months old *A. granulosa* outplants (Baria-Rodriguez et al. 2019). In our experiment, the cost per colony could have been reduced if more corals were outplanted at once, or if the survival rates were higher. For 75% survival of outplants after two years in an optimized-efficiency project initially involving 10'000 fragments, the cost per coral was estimated at 2.34 USD (Edwards et al. 2010), but this is likely to be increased in more realistic settings. Cost estimations largely vary between transplantation studies, ranging from 30 thousand to almost 1.5 million USD per restored hectare (Bayraktarov et al. 2019). In our case, restoring a hectare with five live colonies per m² after two years would approximate to 1.13 million USD. However, costs could be significantly reduced if using sexual propagation instead of coral transplantation. This approach can produce juveniles with an increased survival probability relative to asexually propagated outplants (Baria-Rodriguez et al. 2019). For instance, our costs are substantially higher than the 18 USD required to produce a three-year-old *A. tenuis* via larval enhancement, a process by which millions of larvae are injected in a large net to improve their settlement rate (Harrison et al. 2021). The high cost and labor-intensive act of asexual transplantation is a major drawback of this restoration practice (Ferse et al. 2021), which further highlights the need to optimize cost-efficiency and survival rates of outplants, or invest in more scalable and cost-efficient practices.

## Conclusion

Overall, our transplantation experiment demonstrated a lack of site-dependent effects on coral fitness traits, and this despite the varying environmental conditions of our different sites. In previous studies, more degraded sites characterized by abundant dead corals, sediments and algal competition can significantly reduce survivorship of coral outplants (Dizon & Yap 2006) relative to sites where the planted species naturally occurs (Gomez et al. 2011). The varied environmental conditions in our three sites were likely not different enough to alter outplant fitness, such as similar agal covers and nutrient concentrations at Linareva and Manava. According to our findings, lower coral cover and higher nutrient content are not necessarily disadvantageous for the fitness of outplanted colonies. This suggests that transplantation can be used to successfully restore deteriorated sites, although at a small scale. Further experiments on sites that are even more anthropized than Manava, such as in harbors or sites with high sedimentation rates, could however yield different conclusions.

Instead of site effects, we found strong size-dependent and time-dependent effects linked to the coral's survival, growth, and maturity. This underlines the importance to grow corals in a nursery before outplanting to optimize their survival chance, and to allow a long acclimation time before growth can eventually pick up and more resources allocated towards sexual reproduction. Coral transplantation should not be considered as a miracle solution to save all threatened coral reefs. Climate warming and recurring bleaching events are still the most serious threat to the survival of corals. As such, transplantation should be used to locally restore sites where natural recruitment is limited, combined with effective climate actions and

different restoration techniques that could have more impact at a larger scale, such as larval seeding or assisted evolution.

## Acknowledgements

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# 5. Supplementary materials

Table 3.S1: Statistical tests of different experiments

| Experiment | Test | Response variable | Predictor | $X^2$ (or other stat) | df | p |
|---|---|---|---|---|---|---|
| **Temperature** | Anova | Mean daily temperature | Site | 0.70 | 3 | 0.554 |
| | Anova | Maximum daily temperature | **Site** | **12.69** | **3** | **<0.001** |
| | Pairwise T test | Maximum daily temperature | **Mahana > Linareva + Manava**<br>**Nursery > Manava** | | | |
| | Kruskal | Minimum daily temperature | **Site** | **15.23** | **3** | **0.002** |
| | Pairwise Wilcoxon test | Minimum daily temperature | **Mahana < Manava + Nursery** | | | |
| | Kruskal | Daily range | **Site** | **62.06** | **3** | **<0.001** |
| | Pairwise Wilcoxon test | Daily range | **Mahana > Nursery > Linareva > Manava** | | | |
| **Transects** | Pairwise permanova | Benthic composition | **Linareva vs Mahana** | **26.20** | **1** | **0.003** |
| | | | **Linareva vs Manava** | **21.12** | **1** | **0.005** |
| | | | Mahana vs Manava | 2.94 | 1 | 0.097 |
| | Pairwise permanova | Coral genera composition | **Linareva vs Mahana** | **7.21** | **1** | **0.009** |
| | | | **Linareva vs Manava** | **4.37** | **1** | **0.009** |
| | | | **Mahana vs Manava** | **10.24** | **1** | **0.009** |
| | Kruskal | Live coral cover | **Site** | **11.79** | **2** | **0.003** |
| | Pairwise Wilcoxon test | Live coral cover | **Linareva > Mahana + Manava** | | | |
| | Anova | Algal cover | **Site** | **8.25** | **2** | **0.004** |
| | Pairwise T test | Algal cover | **Linareva + Manava > Mahana** | | | |
| | Anova | Number of coral genera | Site | 3.53 | 2 | 0.056 |
| **Nutrients** | Anova | N content (%) | **Site** | **5.24** | **3** | **0.004** |
| | Pairwise T test | N content (%) | **Manava > Nursery + Mahana + Linareva** | | | |
| **Survival** | Anova(cox) | Survival probability | Transplantation site | 2.35 | 3 | 0.502 |
| | | | Treatment | 0 | 1 | 1 |
| | | | Genotype | 5.79 | 7 | 0.564 |
| | | | **Site*Genotype** | 33.3 | **21** | **0.043** |
| | | | Treatment*Site | 0 | 3 | 1 |
| | | | Treatment*Genotype | 0 | 7 | 1 |
| | | | Treatment*Gen*Site | 0 | 21 | 1 |
| | Anova(cox) | Survival (Transplant) | Site | 2.67 | 3 | 0.445 |
| | | | Genet | 5.79 | 7 | 0.564 |
| | | | **Site*Genet** | **58.46** | **21** | **<0.001** |
| | Anova(cox) | Survival of genotype 2 | **Site** | **10.64** | **3** | **0.014** |
| | Anova(cox) | Survival of genotype 3 | **Site** | **10.82** | **3** | **0.013** |
| | Anova(cox) | Survival of genotype 4 | Site | 1.49 | 3 | 0.686 |
| | Anova(cox) | Survival of genotype 5 | **Site** | **7.95** | **3** | **0.047** |
| | Anova(cox) | Survival of genotype 7 | Site | 7.40 | 3 | 0.060 |
| | Anova(cox) | Survival of genotype 8 | Site | 3.31 | 3 | 0.346 |
| | Anova(cox) | Survival of genotype 9 | Site | 3.01 | 3 | 0.391 |
| | Anova(cox) | Survival of genotype 10 | Site | 2.46 | 3 | 0.483 |
| | Anova(cox) | Survival probability | Site | 4.64 | 3 | 0.200 |
| | | | **Genet** | **33.28** | **7** | **<0.001** |
| | | | Clade A | 0.00 | 1 | 1.000 |
| | | | Clade C | 0.00 | 1 | 1.000 |
| | | | Clade D | 0.00 | 1 | 1.000 |
| | | | **Site*Genet** | **52.69** | **21** | **<0.001** |

| Experiment | Test | Response variable | Predictor | $X^2$ (or other stat) | df | p |
|---|---|---|---|---|---|---|
| **Growth** | Anova(lmer, | Growth (Transplant+Control) | **State** | **152.28** | **5** | **<0.001** |
| | colony as random factor) | | Genotype | 3.65 | 7 | 0.819 |
| | | | Time | 5.66 | 7 | 0.58 |
| | | | Treatment | 0.92 | 1 | 0.337 |
| | | | Initial size | 0.97 | 1 | 0.326 |
| | | | Genotype*Time | 20.1 | 49 | 1 |
| | | | Genotype*Treatment | 0.58 | 7 | 0.999 |
| | | | Time*Treatment | 5.72 | 7 | 0.573 |
| | | | Genotype*Time*Treatment | 7.4 | 30 | 1 |
| | Anova(lmer, | Growth (Healthy) | Initial size | 0.86 | 1 | 0.355 |
| | colony as random factor) | | Genotype | 2.75 | 7 | 0.907 |
| | | | **Time** | **27.74** | **7** | **<0.001** |
| | | | Treatment | 0.79 | 1 | 0.374 |
| | | | Genotype*Time | 35.98 | 48 | 0.899 |
| | | | Genotype*Treatment | 2.21 | 7 | 0.947 |
| | | | **Time*Treatment** | **19.44** | **7** | **0.007** |
| | | | Genotype*Time*Treatment | 26.69 | 30 | 0.639 |
| | Anova(lmer, | Growth (Impaired outplants) | Initial size | 0.03 | 1 | 0.855 |
| | colony as random factor) | | Genotype | 2.02 | 7 | 0.959 |
| | | | Site | 0.99 | 2 | 0.61 |
| | | | **Time** | **32.38** | **7** | **<0.001** |
| | | | **Genotype*Site** | **39.82** | **14** | **<0.001** |
| | | | Genotype*Time | 18.96 | 32 | 0.967 |
| | | | Site*Time | 17.07 | 11 | 0.106 |
| | | | Genotype*Site*Time | 16.69 | 12 | 0.162 |
| | Kruskal | Growth February-March 2021 | **Site** | **11.53** | **3** | **0.009** |
| | Pairwise Wilcoxon | | **Manava < Nursery** | | | |
| | Kruskal | Growth March-June 2021 | **Site** | **22.78** | **3** | **<0.001** |
| | Pairwise Wilcoxon | | **Nursery > Mahana, Manava** | | | |
| | Kruskal | Growth June-October 2021 | **Site** | **20.24** | **3** | **<0.001** |
| | Pairwise Wilcoxon | | **Mahana < Linareva, Nursery** | | | |
| | Kruskal | Growth October-February 2022 | **Site** | **8.70** | **3** | **0.034** |
| | Pairwise Wilcoxon | | NO DIFFERENCES | | | |
| | Kruskal | Growth February-June 2022 | Site | 7.81 | 3 | 0.05 |
| | Kruskal | Growth June-October 2022 | Site | 5.61 | 3 | 0.132 |
| | Kruskal | Growth October-February 2023 | Site | 0.30 | 3 | 0.96 |
| | Kruskal | Growth February-April 2023 | Site | 2.13 | 3 | 0.546 |
| | Kruskal | Yearly growth (Nursery only) | **Year** | **3.92** | **1** | **0.048** |
| | Kruskal | Yearly growth (Transplant only) | **Year** | **6.62** | **1** | **0.01** |
| | Correlation | **Growth (Nursery only)** | **Initial size** | **-2.43** | **105** | **0.017** |
| | Correlation | Growth (Transplantation only) | Initial size | 0.92 | 358 | 0.358 |
| | Correlation | Yearly growth | Percentage of clade A | -0.09 | 139 | 0.267 |
| | | | **Percentage of clade C** | **0.17** | **139** | **0.035** |
| | | | Percentage of clade D | -0.14 | 139 | 0.104 |
| **Maturity** | Binomial GLM + Anova | Maturity in 2021 | **Genotype** | **15.61** | **7** | **0.029** |
| | | | Site | 0.00 | 2 | 1.000 |
| | | | **Size** | **11.46** | **1** | **<0.001** |
| | Binomial GLM + Anova | Maturity in 2022 | **Genotype** | **22.03** | **7** | **0.003** |
| | | | Site | 0.00 | 2 | 1.000 |
| | | | **Size** | **22.29** | **1** | **<0.001** |
| | Binomial GLM + Anova | Maturity in the nursery | Size | 0.63 | 1 | 0.426 |
| | | | **Year** | **4.12** | **1** | **0.042** |

Table S2: Statistical tests of different experiments

| Experiment | Test | Response variable | Predictor | $X^2$ (or other stat) | df | p |
|---|---|---|---|---|---|---|
| **Symbiont diversity** | Permanova (Bray Curtis) | **All symbiont communities** | **Genotype** | **125.78** | **7** | **<0.001** |
| | | | **Treatment** | **9.65** | **1** | **<0.001** |
| | | | **Time** | **71.59** | **2** | **<0.001** |
| | | | **Genotype*Treatment** | **2.37** | **7** | **<0.001** |
| | | | **genotype*time** | **12.68** | **13** | **<0.001** |
| | | | treatment*time | 2.5 | 1 | 0.243 |
| | | | **Gen*Treat*Time** | **1.88** | **3** | **0.02** |
| | Permanova (Bray Curtis) | **Mahana** | **Genotype** | **39.35** | **7** | **<0.001** |
| | | | **Time** | **37.21** | **2** | **<0.001** |
| | | | **Genotype*Time** | **7.29** | **13** | **<0.001** |
| | Permanova (Bray Curtis) | **Manava** | **Genotype** | **42.26** | **7** | **<0.001** |
| | | | **Time** | **47.59** | **2** | **<0.001** |
| | | | **Genotype*Time** | **7.63** | **9** | **<0.001** |
| | Permanova (Bray Curtis) | **Linareva** | **Genotype** | **24.23** | **7** | **<0.001** |
| | | | **Time** | **10.56** | **2** | **<0.001** |
| | | | **Genotype*Time** | **3.04** | **13** | **<0.001** |
| | Permanova (Bray Curtis) | **Nursery** | **Genotype** | **74.87** | **7** | **<0.001** |
| | | | **Time** | **9.43** | **2** | **<0.001** |
| | | | **Genotype*Time** | **4.38** | **9** | **<0.001** |
| | Permanova (Bray Curtis) | **T0** | **Genotype** | **105.15** | **7** | **<0.001** |
| | | | Site | 0.512 | 3 | 0.749 |
| | | | Genotype*Site | 1.08 | 21 | 0.377 |
| | Pairwise Permanova | At T0, all genotypes vs all genotype | | | **All** | **<0.05** |
| | Permanova (Bray Curtis) | **T12+T27** | **Genotype** | **55.5** | **7** | **<0.001** |
| | | | **Site** | **4.7** | **3** | **0.002** |
| | | | **Time** | **29.96** | **1** | **<0.001** |
| | | | **Genotype*Site** | **2.89** | **19** | **<0.001** |
| | | | **Genotype*Time** | **4.62** | **7** | **<0.001** |
| | | | Site*Time | 1.52 | 3 | 0.189 |
| | | | **Gen*Site*Time** | **1.95** | **11** | **0.023** |
| | Permanova (Bray Curtis) | **T12** | **Genotype** | **57.82** | **7** | **<0.001** |
| | | | **Site** | **2.48** | **3** | **0.047** |
| | | | **Genotype*Site** | **2.02** | **19** | **0.01** |
| | Permanova (Bray Curtis) | **T27** | **Genotype** | **6.03** | **7** | **0.018** |
| | | | Site | 3.03 | 3 | 0.079 |
| | | | Genotype*Site | 1.93 | 11 | 0.184 |

**Table 3.S3.** Pairwise comparisons of symbiont communities at T0 in the nursery in each genotype.

| Observed statistic | Free Stepdown Adjusted P-Value | comparison |
|---|---|---|
| 26.66054389 | 0.001 | 2 vs 8 |
| 25.76529726 | 0.001 | 3 vs 5 |
| 25.7220598 | 0.001 | 3 vs 8 |
| 25.4296211 | 0.001 | 3 vs 9 |
| 24.42801416 | 0.001 | 10 vs 3 |
| 24.29793051 | 0.001 | 2 vs 9 |
| 24.07143782 | 0.001 | 10 vs 2 |
| 21.17341966 | 0.001 | 2 vs 5 |
| 12.63772619 | 0.001 | 2 vs 4 |
| 12.49713983 | 0.001 | 7 vs 8 |
| 12.32889011 | 0.001 | 4 vs 5 |
| 12.03496305 | 0.001 | 7 vs 9 |
| 11.87989068 | 0.001 | 10 vs 7 |
| 11.82852538 | 0.001 | 5 vs 7 |
| 11.09446307 | 0.001 | 4 vs 9 |
| 10.9753305 | 0.001 | 10 vs 4 |
| 10.63840998 | 0.001 | 4 vs 8 |
| 10.03940592 | 0.001 | 2 vs 7 |
| 7.984027551 | 0.001 | 2 vs 3 |
| 6.085737804 | 0.001 | 3 vs 4 |
| 5.334140302 | 0.001 | 3 vs 7 |
| 2.581124677 | 0.001 | 5 vs 8 |
| 2.108928232 | 0.001 | 10 vs 5 |
| 1.582984921 | 0.001 | 10 vs 8 |
| 1.269238584 | 0.001 | 5 vs 9 |
| 1.246728707 | 0.001 | 10 vs 9 |
| 1.028938281 | 0.001 | 4 vs 7 |
| 0.598463095 | 0.001 | 8 vs 9 |

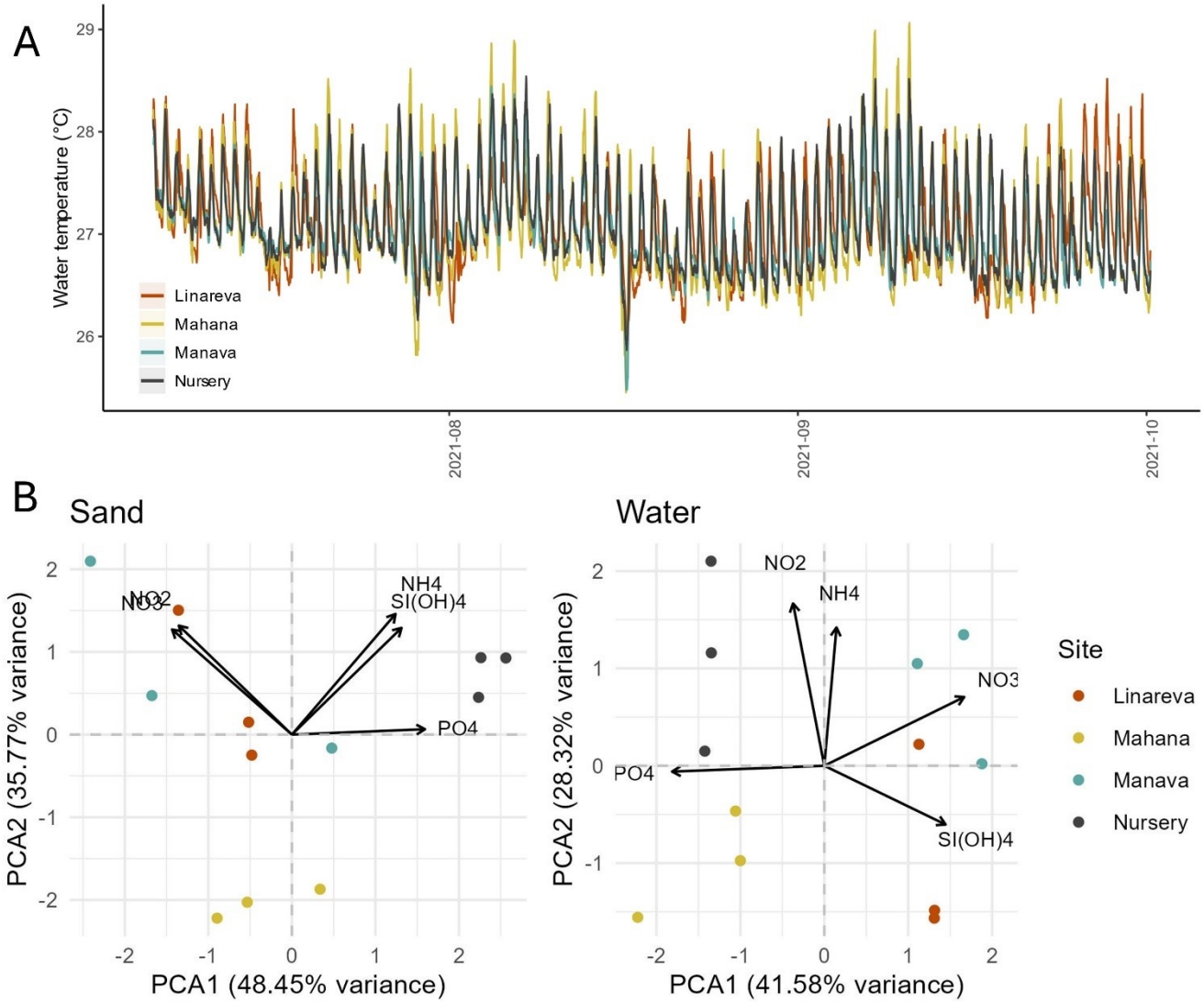



**Figure 3.S1.** Temperature every 30 minutes in four sites in the lagoon of Mo'orea over three months in 2021 (A). Nutrient concentrations in sand and water samples by site (B).

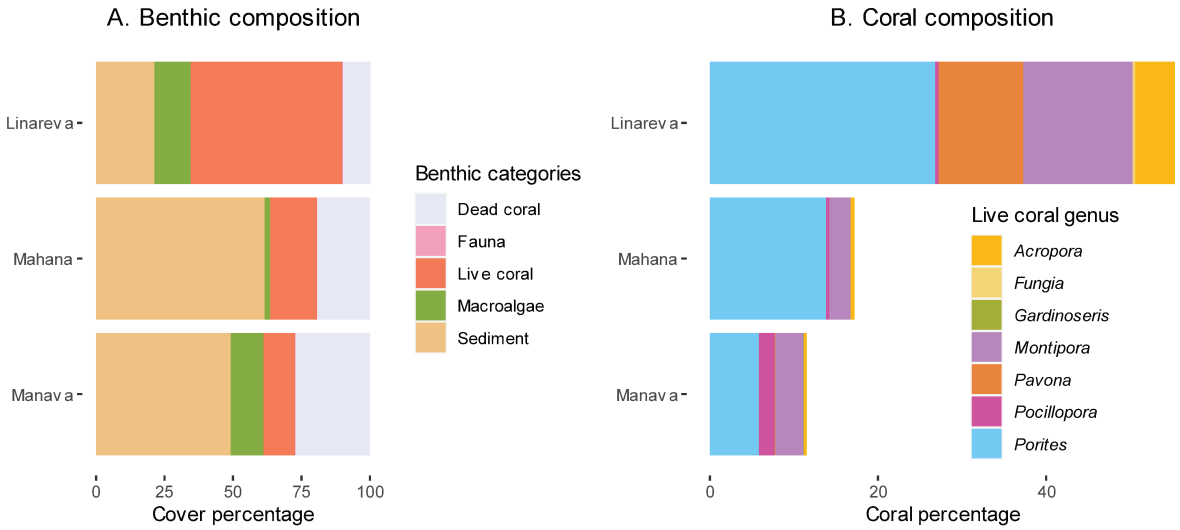



**Figure 3.S2.** Composition of benthic cover of transplantation sites in the lagoon of Mo'orea (A), and genera of live corals in each site (B)

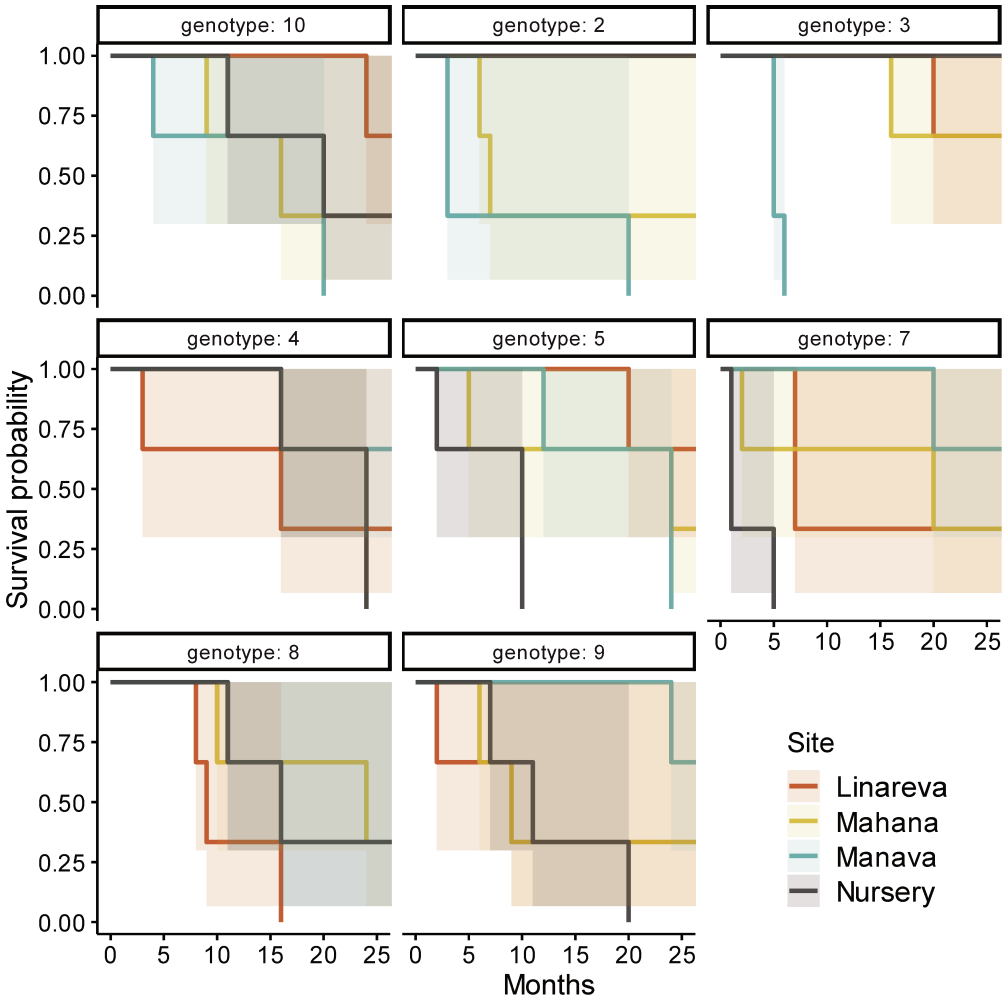



**Figure 3.S3.** Survival probability of *Acropora cytherea* colonies transplanted to different lagoon sites, or kept in the suspended coral nursery.

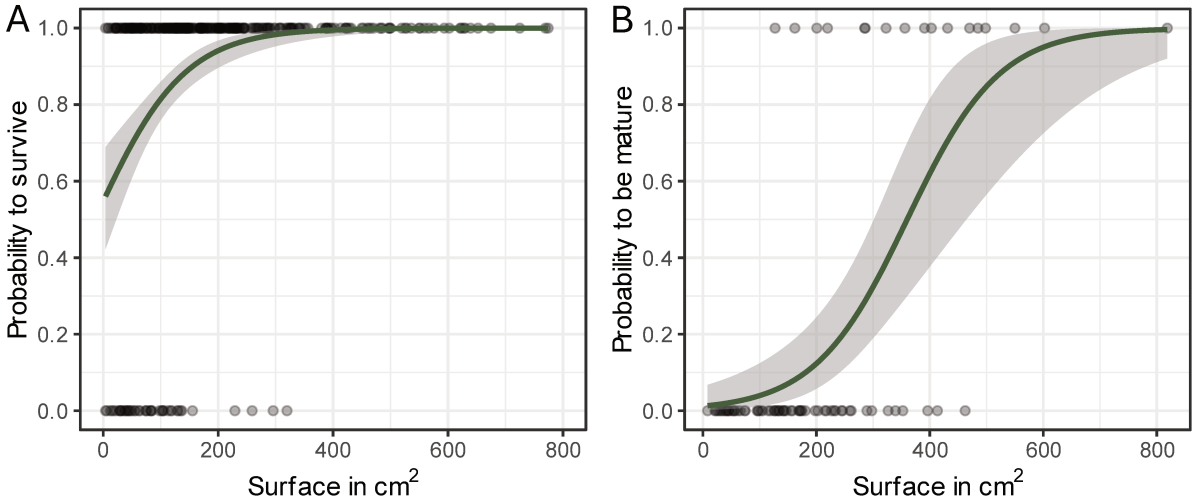



**Figure 3.S4.** Binomial regression of the probability of transplanted *Acropora cytherea* colonies to survive (A) or to be mature (B) according to their size (surface in $cm^2$).

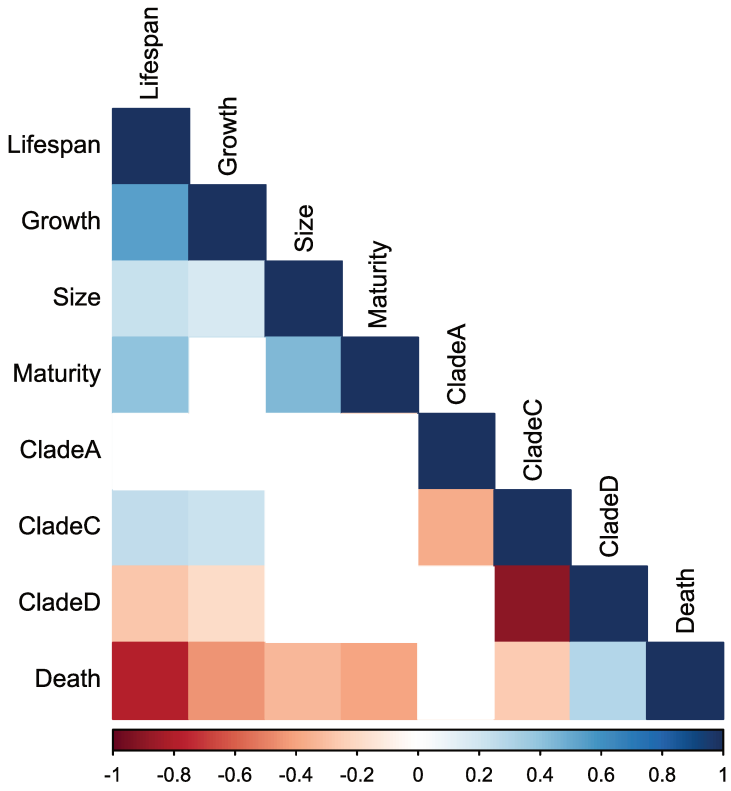



**Figure 3.S5.** Correlation plot of *A. cytherea* death probability (death observed over 27 months), lifespan (months alive), growth rate (percent per day), size (surface in $cm^2$), maturity probability 20 months after transplantation, and symbiont diversity (percent of clades A, C and D). Only significant correlations with pval < 0.05 are shown.

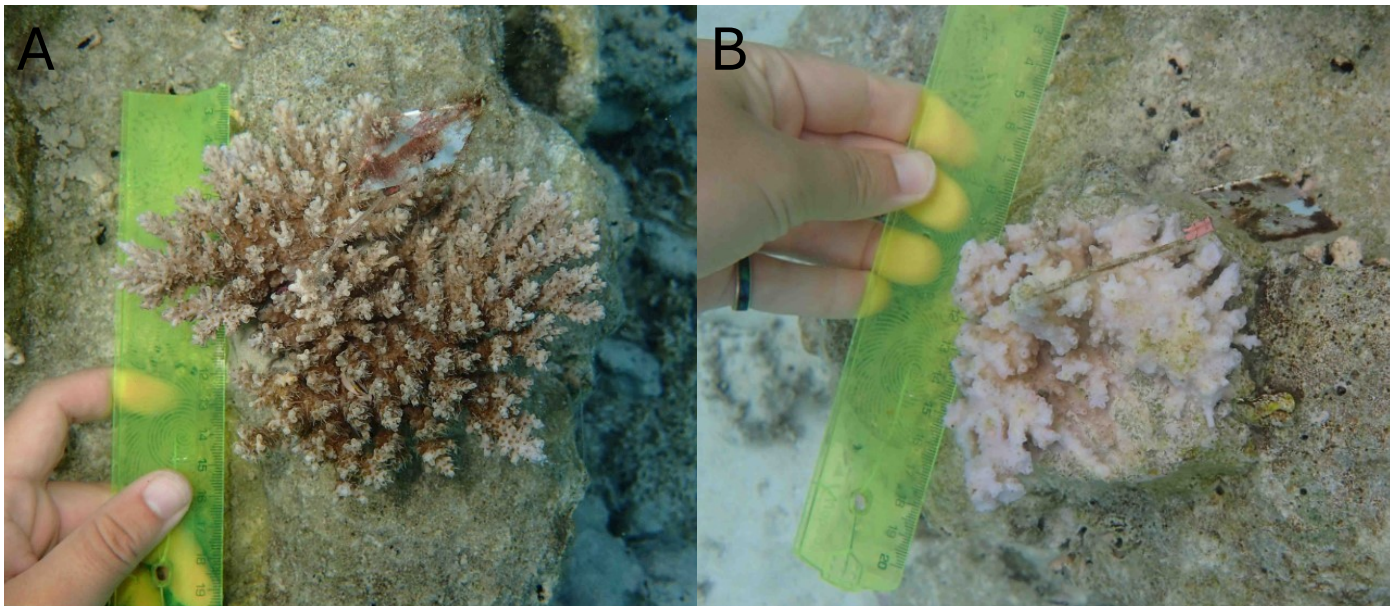



**Figure 3.S6.** Representative photo of a nursery-reared *A. cytherea* colony at transplantation (A) and the same colony one month later, showing signs of predation (B).

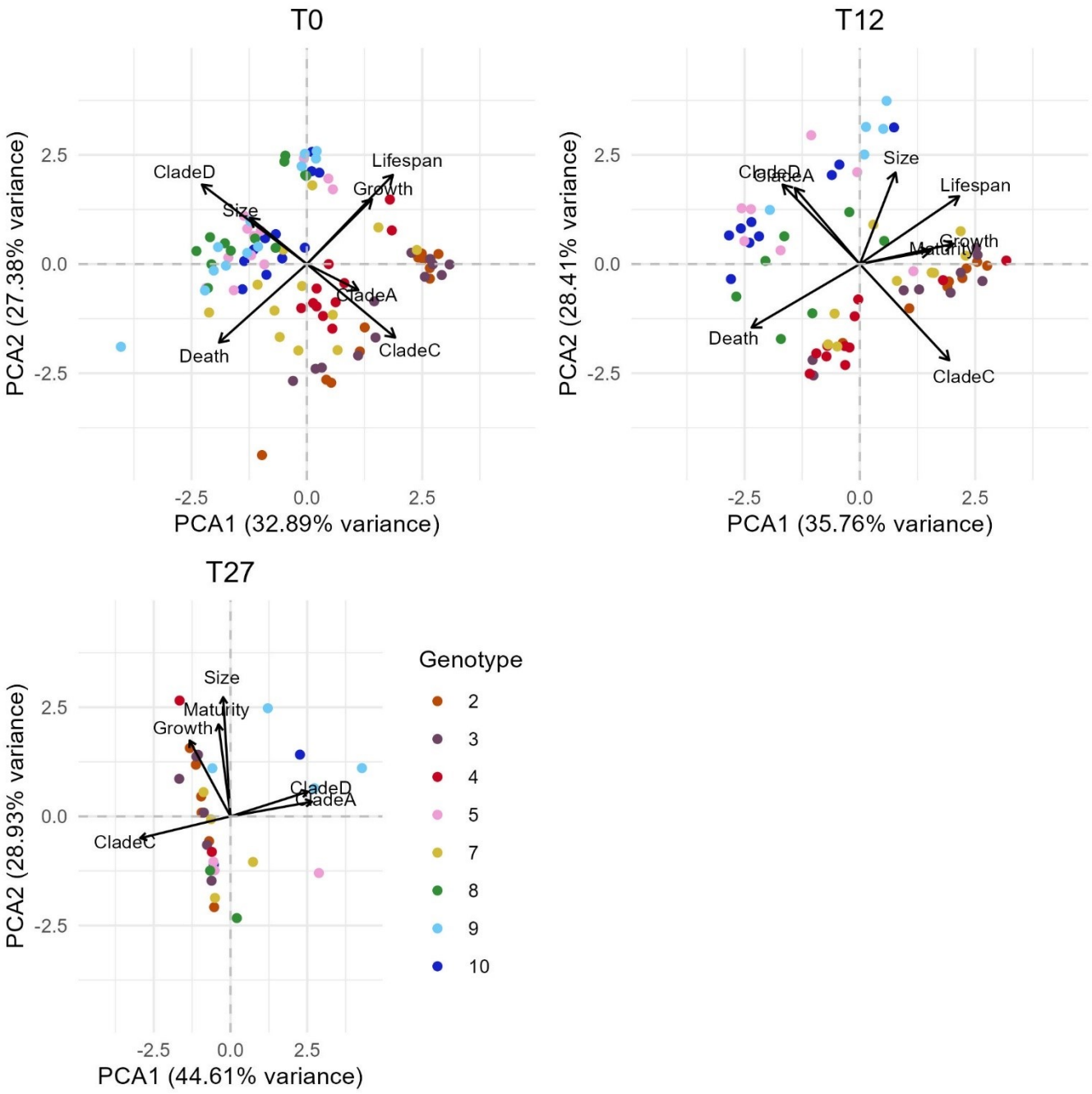



**Figure 3.S7.** PCA of *A. cytherea* growth rate, size, mortality, maturity, and symbiont diversity depending on genotype and time (0-27 months after transplantation).

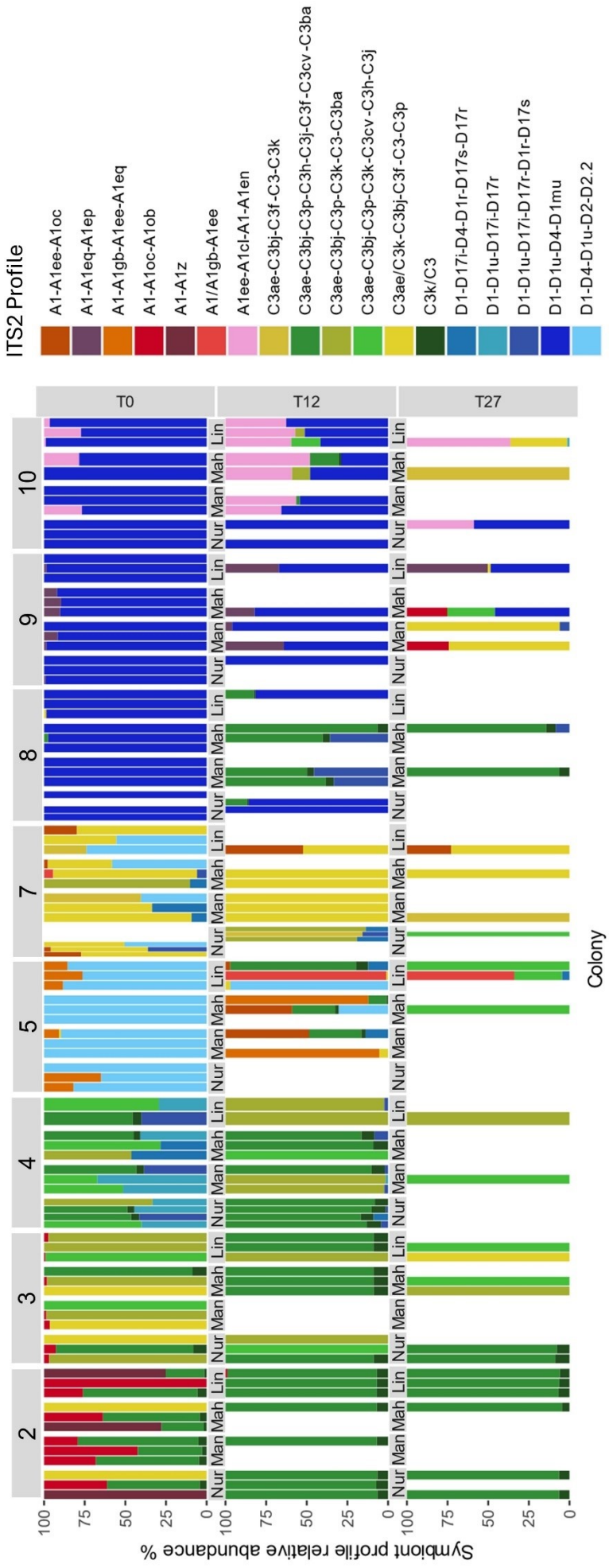



**Figure 3.S8.** Predicted major ITS2 type profiles (SymPortal) of eight different genotypes (2-10) of *A. cytherea* in a lagoon nursery at T0, then 12 and 27 months after transplantation to three different lagoon sites (T12 and T27): Nur = nursery, Man = Manava, Mah = Mahana, Lin = Linareva. Only profiles with more than 1% relative abundance are shown.

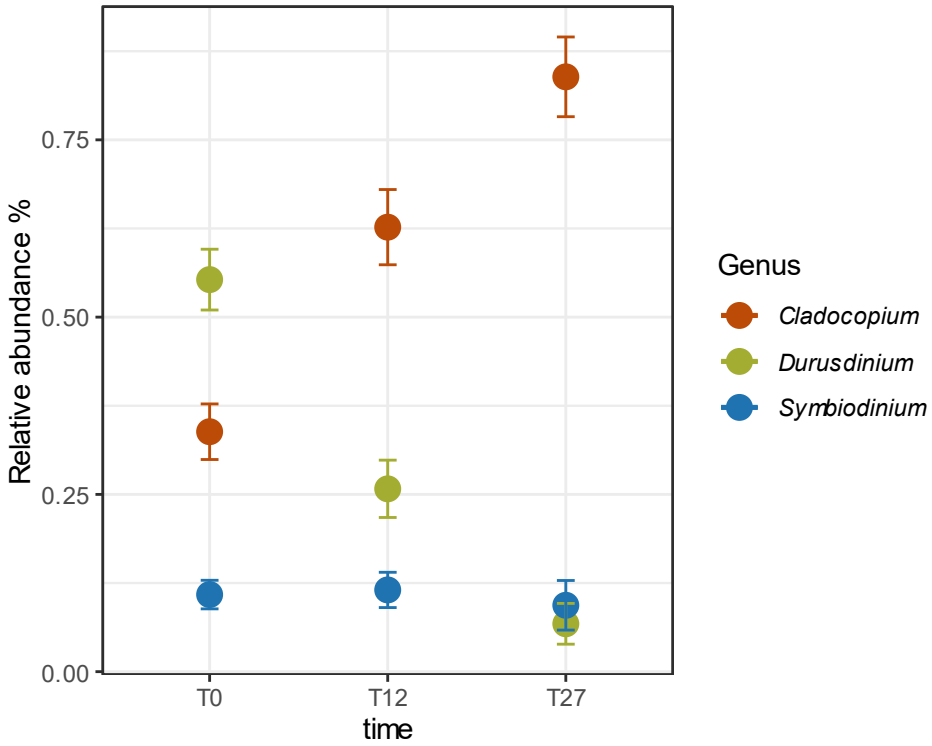



**Figure 3.S9.** Evolution of the relative abundance in Symbiodiniaceae genera over 27 months after the transplantation of the *A. cytherea* host. Error bars represent the standard error of the mean.

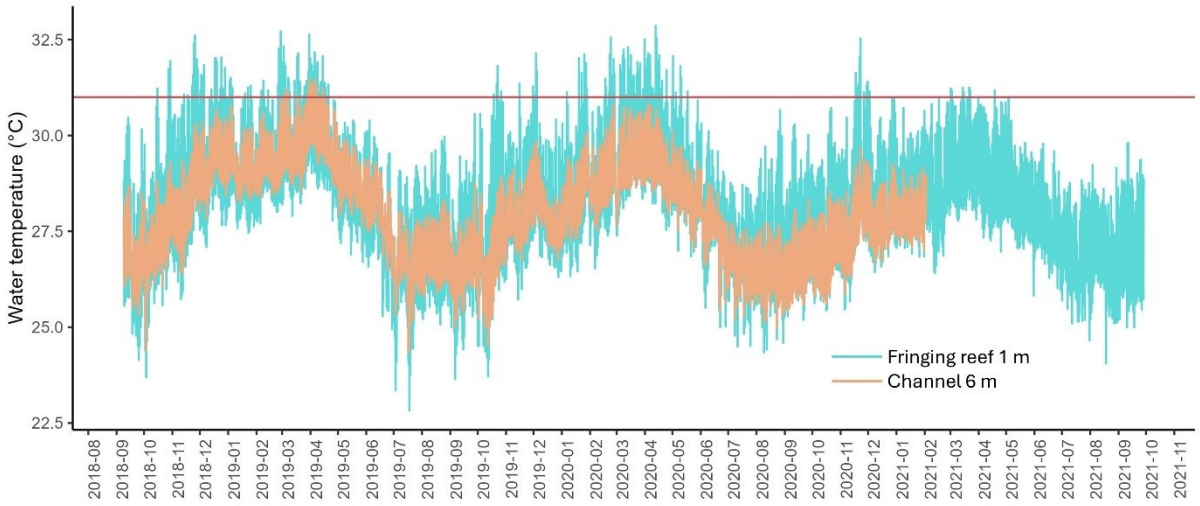



**Figure 3.S10.** Temperature at Mahana fringing reef and deeper channel between 2018 and 2021. The red line indicates the estimated bleaching threshold at 31°C.

# Chapter 4

## Enhancing *Acropora* sp. coral settlement: Insights from wild and nursery-reared spawning patterns and larval seeding strategies

# Enhancing *Acropora* sp. coral settlement: Insights from wild and nursery-reared spawning patterns and larval seeding strategies

**Running head:** Spawning patterns and larval enhancement

*Camille Leonard[1], Danielle Becker[2], Dennis Conetta[2], Julie Stojakovic[1], Yann Lacube[1], Laetitia Hédouin[1,3]*

[1] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, BP 1013, 98729 Papetoai, Mo'orea, French Polynesia

[2] Department of Biological Sciences, University of Rhode Island, Kingston, RI, USA

[3] Laboratoire d'Excellence "CORAIL", Papetoai, Mo'orea, French Polynesia

## Abstract

With growing anthropogenic pressures endangering coral reefs worldwide, coral restoration practices using both sexual and asexual reproduction are being developed as means to increase coral cover and reef resilience. As nursery rearing methods remove corals from their natural environment, it can potentially alter the spawning patterns and reproductive output of coral fragments. In this study, we better apprehend the sexual reproduction of five nursery-reared *Acropora* species (*Acropora cytherea*, *A. hyacinthus*, *A. nasuta*, *A. pulchra* and *A. retusa*) over the spawning seasons of 2020 until 2022. First, we compared their reproductive ecology to that of wild populations. Results showed that the spawning day and time was the same between nursery-reared and wild populations for three (*A. hyacinthus*, *A. nasuta*, *A. retusa*) species. By contrast a slight delay was noted for nursery *A. pulchra* and *A. cytherea* compared to wild population. The egg size, eggs per bundle and per cm², and fertilization rates at 27 and 31°C did not vary between populations, except for *A. retusa*. To better apprehend the role of parent colony in successful fertilization, we performed a cross-specific experiment and revealed that the fertilization rates at 31°C could be enhanced up to 91-fold when crossing highly compatible nursery-reared genotypes of *A. cytherea* compared to less compatible crosses. Parents with the same dominant symbiont genus showed higher compatibility at 31°C, and overall fertilization was enhanced for nursery-reared parents containing more *Symbiodinium* sp. and *Cladocopium* sp. than *Durusdinium* sp. Finally, we tested assisted larval enhancement for *A. cytherea* and noted significant increases in recruitment rates in a degraded lagoon. Our experiment is a proof of concept that larval enhancement can be implemented by a large panel of users using accessible equipment and techniques, even in an artisanal way. The presented experiments address knowledge gaps still limiting the optimization of restoration practices using both sexual and asexual reproduction.

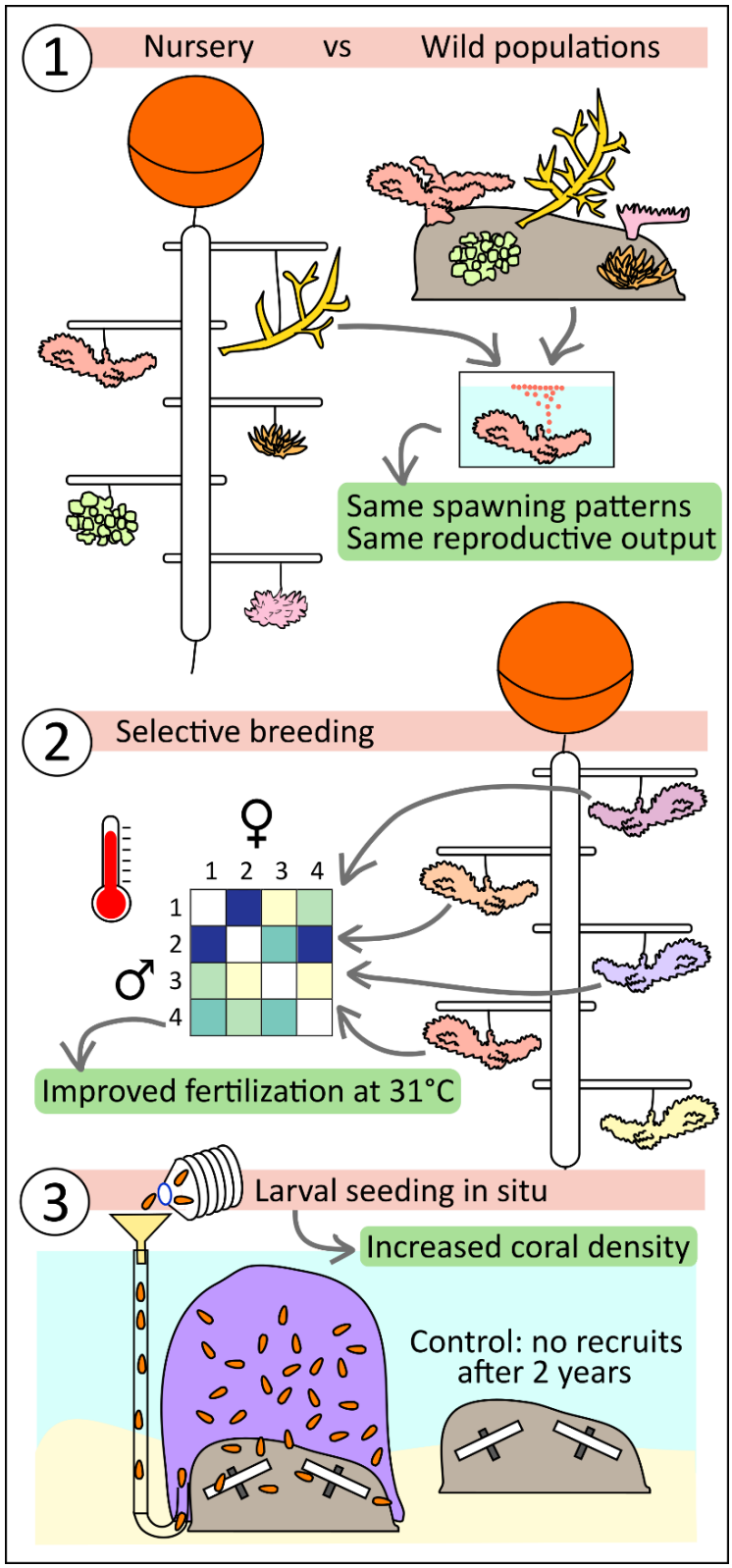



**Figure 4.1.** Graphical abstract of "Enhancing *Acropora* sp. coral settlement: Insights from wild and nursery-reared spawning patterns and larval seeding strategies". Part 1 investigates the reproductive timing and output of conspecific wild colonies and nursery-reared colonies. Part 2 tests the potential of individual crosses between nursery genotypes of *A. cytherea* to improve fertilization rates at ambient and high temperatures. Part 3 hypothesizes that coral recruitment can be durably improved in degraded sites through seeding of ex situ obtained larvae.

# 1. Introduction

Tropical coral reefs play a vital role in safeguarding coastal areas and supporting the livelihoods of millions of people. They are ecologically vital for marine biodiversity and essential nurseries for diverse fish populations (Woodhead et al. 2019). Reef-building corals are able to thrive in nutrient-poor waters due to their obligate endosymbiosis with photosynthetic algae of the Symbiodiniaceae family (Muscatine & Cernichiari 1969). Unfortunately, coral reefs are critically threatened by anthropogenic pressures, in particular global climate change (Hoegh-Guldberg et al. 2018). With growing pressures endangering coral reefs worldwide, interventions aimed at increasing coral abundance and resilience are becoming more common. Since the late 1980's, the asexual propagation of corals has been used to multiply and grow fragments through 'coral gardening', then transplanting them to degraded sites (Guzmán 1991; Rinkevich 1995; Bowden-Kerby 1997). Restoration techniques now range from indirect methods by removing local stressors (pollution, competition, predators), to enriching the substrate through artificial structures, or more direct methods such as the spread of adult corals or sexually produced juveniles or larvae, and the artificial enhancement of coral resistance (McLeod et al. 2022).

Raising corals in in situ nurseries can have various positive or negative impacts on their health and physiology. For example, building nurseries that have protection from strong wave action will enhance coral survival rates (Baria-Rodriguez et al. 2019). Also, suspending fragments on a string can significantly increase coral growth rates (Lirman et al. 2014; O'Donnell et al. 2017), likely through a reduction of sedimentation and competitors (Rinkevich 2014). Further, corals skeletal structure is impacted when suspended and coral fragments typically develop a less dense and more fragile skeleton (Kuffner et al. 2017). A higher growth rate can potentially lead to a better size-dependent survival rate (Raymundo & Maypa 2004; Humanes et al. 2021) and a faster production of mature colonies than in natural conditions (Álvarez-Noriega et al. 2016). However, there are potential trade-offs for an increased growth, for instance a compromised immune response (Schlecker et al. 2022). Additionally, a change of environment can also alter the growth and survivorship of coral fragments (Howlett et al. 2021), for instance when moved between lagoon and fore reef (Dehnert et al. 2022). A change in light regime, to a deeper or shallower nursery, can modify the symbiont density and photosynthetic activity (Cohen & Dubinsky 2015; Tamir et al. 2020). These diverse changes in physiological capacities will in turn likely influence the energetic reserves allocated to reproduction.

Few studies have investigated how in situ nursery rearing can alter the sexual reproduction of corals (Amar & Rinkevich 2007; Zayasu & Suzuki 2019). The stress of fragmentation and change of environment could potentially limit corals reproductive capacity, leading to changes in egg size and fecundity (Lirman 2000; Zakai et al. 2000; Kai & Sakai 2008). When fragmented from mature colonies, *A. hyacinthus* fragments as small as 4 cm can retain the ability to spawn the following spawning season (Rapuano et al. 2023). However, this can strongly depend on the timing of the fragmentation; if done before or during early vitellogenesis, coral fragments will regress to an immature state and reproduction can be inhibited for up to three years (Okubo

et al. 2007). Coral fragments then need to reach a certain size threshold to become mature again (Hall & Hughes 1996), but the size and age can greatly vary between species. This can be only two years in Pocilloporid fragments (Amar & Rinkevich 2007), but much longer for slow-growing species (Babcock 1991). A nursery setting can also be beneficial for the rapid maturity onset in *Acropora millepora* or *Stylophora pistillata* (Amar & Rinkevich 2007; Baria et al. 2012) and nurseries can represent artificial spawning hotspots thanks to a high density of compatible corals producing significantly more gametes than wild populations (Amar & Rinkevich 2007; Zayasu & Suzuki 2019). This improved reproductive output could be linked to nutrient-enriched waters in some sites (Bongiorni et al. 2003). Similarly, aquarium rearing can improve the egg size, fertilization and survival rates of *A. intermedia* (Okubo et al. 2010). As the results concern only a few species, there is still a lack of consensus on whether nurseries significantly alter the reproduction of corals relative to that of wild populations.

While asexual reproduction through fragmentation and parthenogenesis is common in some coral species (Ayre & Miller 2004), sexual reproduction is crucial for maintaining genetic diversity and for long-distance dispersal (Harrison 2011). Restoration using only asexual fragmentation presents the risk that using clones of a few genotypes does not contribute to an improved genetic diversity, and thus limits the resistance potential to future perturbations (Baums et al. 2022). Recommendations are now to combine both sexual and asexual practices in order to maximize beneficial outcomes (Randall et al. 2020). Restoration using sexual reproduction is now becoming a promising scalable method for improving long-term coral cover (Doropoulos et al. 2019a; Randall et al. 2020). As transplantation of adult colonies can prove to be time-consuming for few surviving colonies, sexually produced corals can show a faster growth and increased survival rate, thus a better cost effectiveness (Baria-Rodriguez et al. 2019). Concentrated spawning hotspots can also potentially restore reefs more efficiently than transplanting a large number of mature corals (Zayasu & Suzuki 2019). With increasing research efforts, restoration experiments using sexual reproduction are becoming more perfected, spanning all the way from assisted evolution (Van Oppen et al. 2015), interspecific hybridization (Chan et al. 2018), thermal priming of gametes (Puisay et al. 2023), crossing of heat-resistant populations (Quigley et al. 2020; Howells et al. 2021), to the widespread larval seeding of entire damaged reefs (Doropoulos et al. 2019a). While most of these practices are still at the experimental stage, they represent a potential in significantly increasing larval supply, which is essential for species recovery, and increasing genetic diversity and the resilience of reefs in the face of future perturbations.

Selective breeding is an aspect of coral restoration that aims to cross parents that could produce offspring with desired qualities, such as heat resistance (Quigley et al. 2020; Howells et al. 2021), as heat stress is currently the most pressing threat to coral reefs (Hoegh-Guldberg et al. 2018). Selective breeding can produce different fertilization rates based on the compatibility of crossed corals (Willis et al. 1997; Miller et al. 2018; Koch et al. 2022b). Like most corals, *Acropora* are hermaphrodite but usually self-incompatible (Heyward & Babcock 1986; Willis et al. 1997; Fogarty et al. 2012), thus finding a compatible sexual partner is crucial

for successful fertilization outcomes. Genotype compatibility can result in faster-swimming larvae even at elevated temperatures (Baums et al. 2013), therefore producing offspring with an increased heat resistance. Various experiments have shown strong parental effects in the heat-tolerance of the offspring, such as increased larval survival when parents came from warmer reefs (Dixon et al. 2015; Drury et al. 2021; Johnston et al. 2024). A better understanding of such parental effects would allow optimizing offspring survival and fitness, then select larvae with enhanced capacities to improve the success of other restoration practices, such as larval seedings.

As a single coral colony can produce huge quantities of gametes (up to 260 eggs per $cm^2$ of coral tissue) (Wallace 1985), once ex situ spawning or in situ collection with floating nets is mastered (Suzuki et al. 2020), obtaining large quantities of coral slick is relatively uncomplicated. Protecting embryos in a rearing tank or a floating net can reduce the very high mortality of larvae encountered through predation in the ocean (Connolly & Baird 2010; Suzuki et al. 2020). Once they are ready to settle, coral larvae can be used to seed specific artificial substrates or certain degraded areas of reefs where natural settlement is low, through assisted larval enhancements. To this day, less than ten larval enhancement projects have been published, using mostly *Acropora* larvae and one study using *Porites astreoides* larvae (Cooper et al. 2014). Some yielded very promising recruit densities at first, but were not monitored long-term, or showed very high post-settlement mortality (Heyward et al. 2002; Cooper et al. 2014; Edwards et al. 2015). One study showed significantly improved recruitment even in an anthropized port (Omori et al. 2003), and some benefitted from artificial materials improving survival rates (Suzuki et al. 2011; Cameron & Harrison 2020). Three studies seeded up to 1.5 million larvae on large plots up to 25 $m^2$, which produced hundreds of mature colonies and an enhanced fish abundance after only three years (dela Cruz & Harrison 2017, 2020; Harrison et al. 2021). Depending on the long-term survival and prevalence of natural recruitment, larval seeding could be a scalable way to restore large areas, and thus more cost-effective than transplanting adult colonies (Doropoulos et al. 2019a).

The aim of the present paper was to evaluate the impact of nursery rearing on the reproductive output of corals, and to trial two ways to use sexual reproduction for coral restoration. Raising corals in nurseries can alter their health and physiology, and potentially their reproductive behavior, but very few studies compared the reproduction of wild versus nursery corals. Considering the natural setting of in situ nurseries, the environmental cues influencing spawning patterns should be the same as for wild populations. Thus, we hypothesized that nursery rearing would not alter the timing of spawning. We also expected the overall reproductive output of nursery-reared corals to remain unchanged relative to that of wild conspecifics. In that case, nursery-reared corals could be used to restore damaged reefs and contribute to the local spawning populations by remaining fertile and synchronized.

Considering the significant advantages of restoration using sexual reproduction, we decided to test two case studies on *Acropora cytherea*, a large tabular species in Pacific lagoons, that is often underrepresented in restoration projects. Capitalizing on the presumed varying

genotype compatibilities, we tested whether fertilization rate could be optimized at elevated temperatures by selecting specific pairs of parents, in order to produce offspring with an improved resistance. Finally, we hypothesized that we could significantly enhance coral recruitment in degraded areas by assisting the settlement of larvae obtained during ex situ spawning events. Larval seeding has been trialed on a few *Acropora* species, but never on *A. cytherea,* which is an important structural species that is often lacking in degraded lagoons. The tools previously used for larval seeding were very large nets with important quantities of larvae. Our goal was to test whether larval seeding could be successfully implemented at a smaller scale, with a lesser budget and simpler tools. If such restoration project can be implemented with easy steps and small means and still show positive results, they could potentially be implemented worldwide and by a large panel of users, even in an artisanal way. These experiments address some of the knowledge gaps still limiting the optimization of restoration practices (Randall et al. 2020).

# 2. Methods

## 2.1 Experiment 1: Spawning pattern of wild versus nursery-reared *Acropora* sp.

### 2.1.1 Coral sampling

Mature colonies, characterized by colored oocytes, from wild populations of *Acropora pulchra*, *A. hyacinthus*, *A. cytherea*, *A. nasuta* and *A. retusa* were collected around Mo'orea, French Polynesia, a few days following the full moons of October and November 2020. Colonies from the nursery population had been growing for at least two years in a suspended lagoon nursery at 5-7 m depth, in the channel of Pihaena (map and GPS coordinates of each site in Fig. 4.S1). Wild *A. pulchra* were collected in the lagoon at 1 m depth (Linareva, Mahana, Manava). Wild *A. cytherea* were collected in the lagoon at 1 m depth (Linareva and Teavaro). Wild *A. hyacinthus*, *A. retusa* and *A. nasuta* were collected on the fore reef, at 10 m depth. Colonies were kept for up to two weeks in 300L flow-through filtered seawater tables, outside with no artificial lighting, as this is known to delay the spawning (Davies et al. 2023). Following the spawning, they were planted back to their site of origin. The size of corals was estimated with a photo of their largest surface, next to a ruler for scale. Photos were analyzed on ImageJ (Schneider et al. 2012) and the surface was measured in $cm^2$ with the freehand polygon selection tool. If corals were fragments of larger wild colonies, their size was not recorded. *A. pulchra* were not measured, and neither were wild *A. cytherea* as these were too large. While all species were studied in 2020, *A. cytherea* populations were also surveyed in the same way for the 2021 and 2022 spawning.

### 2.1.2 Timing

Full moon and sunset times in Tahiti were gathered from "timeanddate.com". The day of spawning was expressed as the number of days after the full moon (AFM). The time of

spawning was expressed as the time after the sunset in Tahiti and was noted precisely for *A. cytherea* and *A. retusa*, but only approximations are available for other species.

### 2.1.3 Fecundity

An hour before spawning, colonies were isolated in 30-50 L seawater tanks, without water flow. A 2-3 cm fragment was cut and placed in a clean 100 ml sample jar, with just enough seawater to cover the fragment. After spawning, the jar was filled with twice the seawater volume in formaldehyde 10% (final concentration 6.7%). Later, the eggs were washed and photographed using a Leica stereomicroscope. They were counted using ImageJ Cell Counter. The fragments were rinsed then bleached in 8° bleach for 24 hours, then washed and airdried for 48 hours. The fragments were then scanned using the EinScan SP scanner from SHINING 3D. The maximum length was noted, the width, the total surface and surface of live tissue (total surface – surface of cut), and total volume. The fecundity index was the total number of eggs released divided by the surface of live tissue in $cm^2$.

### 2.1.4 Eggs per bundle

During spawning, 24 random bundles were collected in 24-well plates for each colony. Eggs were counted under a stereomicroscope for each bundle, one hour after spawning.

### 2.1.5 Egg size

Bundles were collected in clean falcon tubes for each colony separately. Bundles were gently broken up by shaking, then filtered on a 150 µm mesh, and the eggs were washed four times with filtered seawater to remove any sperm. A clean 1.5 ml tube was filled with 1/5 eggs and seawater and 4/5 formaldehyde 10% (final concentration 8%). Another day, formaldehyde was washed away, and eggs were photographed with a Leica stereomicroscope. Then, 50 random eggs were measured using ImageJ software. Egg size was the mean of the longest length and the largest perpendicular width.

### 2.1.6 Fertilization rate

All bundles were collected in 50 ml falcons in seawater. After 30 minutes, bundles had naturally broken apart or were gently shaken. Eggs and sperm were separated on a clean 150 µm filter, then eggs were washed four times with filtered seawater (Whatman® glass microfiber filters, binder free, Grade GF/C circles, 47 mm diameter and 1.2 µm pores). Sperm concentration was measured via spectrophotometer (750 nm). About 200 eggs were added to 10 mL vials of filtered seawater, then the sperm was added to obtain $1*10^6$ cells per mL in the 10 mL vial. Vials were incubated in a large seawater tank where the temperature was kept at either 27°C (± 0.1°C) or 31°C (± 0.1°C) with aquarium heaters. Each cross was replicated five times. After three hours, embryos were transferred with a plastic pipette to a 1.5 ml tube filled with 96% ethanol to stop their development. Later, embryos were laid out on a Petri dish, photographed with a stereomicroscope and all embryos were counted on ImageJ and classified as either unfertilized (no division), ‘normal fertilized’ (even divisions) or ‘deformed fertilized’ (abnormal

deformations). Fertilization rate was expressed as the number of normal fertilized embryos in one tube divided by the total number of embryos.

## 2.2 Experiment 2: Nursery *A. cytherea* selective breeding

### 2.2.1 Individual crosses

Nursery-reared *A. cytherea* were selected for a case study on the potential of optimizing fertilization rates with selective breeding. Fertilization success of individual two-parent crosses (one egg donor and one sperm donor) was tested on nursery-reared *A. cytherea*, over three spawning seasons (2020-2022), with 8 separate genotypes (because of a successful fertilization). Two crosses showed no fertilization (genotypes 1x4 and 3x20); thus, genotypes were assumed to be identical and grouped in all analyses.

### 2.2.2 Symbiont diversity

Symbiont DNA was extracted for a separate experiment on different colonies than those used for spawning, but belonging to the same genotypes. Exact sampling, extraction and analysis are detailed in Chapter 3. Briefly, twelve corals of 8 genotypes in the nursery were sampled in February 2021, DNA was extracted with a Zymo Quick-DNA™ kit, amplified with a first PCR using the primers SYM_VAR_5.8S2 and SYM_VAR_REV (Hume et al. 2018), sequenced on Illumina MiSeq and analyzed with SymPortal (Hume et al. 2019). Because each genotype had their own unique symbiont communities, abundances of the 20 most abundant strains were averaged for each genotype and assigned to the genotypes used in the present study.

## 2.3 Experiment 3: Larval seeding

### 2.3.1 Larval production

*A. cytherea* was selected for a case study on enhanced larval settlement. Ten fragments of *A. cytherea* (approximately 20 cm long) were collected on 26th of September 2021 at 1 m depth on the back reef of Linareva (17°33'13.4"S 149°53'08.5"W) in Mo'orea, French Polynesia. Their maturity was checked prior to collection by examining the color (pink) of egg bundles inside the coral polyps. They all spawned six days AFM at 10 pm at the CRIOBE research station. All the gametes were pooled, left to rest for an hour until complete fertilization, then the excess sperm was washed away, and the embryos transferred to a 300 L filtered sea water tank. After two days without water exchange, a low water and air flow were added to the tank. Six days after fertilization, larvae were actively swimming in the water column and ready to settle. The larvae were then concentrated in a 5 L container using a 150 µm mesh strainer. Three times 10 ml were sampled and counted to estimate the concentration of larvae. Six 5 L plastic bottles were then filled with 12,000 larvae each. These were transported to the lagoon site on a boat in an isotherm box.

### 2.3.2 Experimental setup

The selected experimental site was Mahana (Fig. 4.S1), at 1.5 m depth, on the fringing reef on the north coast of Mo'orea. Twelve bare bommies (with less than 5% live encrusting corals) were selected for the experiment. Each was photographed from above with a ruler, the surface outlined on ImageJ and the total surface was approximated to the surface of a hemisphere (surface of circle x 2). They were carefully surveyed for existing *Acropora* recruits, and only one *A. hyacinthus* recruit was noted.

In each bommie, three holes were drilled with a Nemo underwater hammer drill, and plastic wall plugs were attached, which held the settlement tiles in place. These were made of clean 10x11 cm rigid foam boards, each carrying 8 plastic settlement plugs. The plugs were previously conditioned for eight weeks at 14 m depth on the forereef. They were positioned face-down on the lower side of the board, because coral settlement is increased on lower sides and in cryptic habitats.

Six bommies were covered by nets. The nets were shaped like cylindrical tents (approximately 1 m diameter), sewn with 150 µm mesh, with at the bottom a fabric hem carrying a 3 m long steel chain. Within the top of the net, a slightly inflated bicycle tube kept the net upright.

Approximately 12,000 larvae were injected into each of the six nets, by pouring the bottles in a plastic hose and funnel from the surface. After 48 hours, the nets were removed from the bommies. After ten days, all settlement plugs were retrieved to count the natural vs. seeded recruits. (They were not replaced because plastic settlement plugs proved unfit for survival: recruits easily slid off the smooth surface and would not have survived long-term in situ). The bommies were tagged, and monitored for recruits after 12 months, 16 months and 21 months.

## 2.4 Statistical analysis

Data are reported as mean values ± standard error. A Shapiro-Wilk test was used to test the normality of all the data and to determine the need for parametric or non-parametric tests and models. The influence of species and population on the day of spawning (days AFM) was tested using negative binomial generalized linear mixed effects models (*glmer*) function from the *lme4* R package, with genotype as a random factor, with the *Anova* and a negative binomial family from the *car* and *glmmTMB* packages (Bates et al. 2007, 2015; Brooks et al. 2017). Model fit was tested with the random distribution of the residuals vs. fitted plot. The influence of the population on the day and the time (minutes after sunset) of spawning of each species separately was analyzed with a Kruskal-Wallis test. The probability to show split spawning (over multiple nights) was analyzed with a binomial *glmer*, with the species and population as explanatory variables and genotype as a random factor. The significant differences between species were tested with a pairwise Wilcoxon test. The influence of species and population on fecundity and on the number of eggs per bundle was tested with a negative binomial *glmer* with genotype as a random factor, and differences between species were tested with a pairwise Wilcoxon test. The same was done for egg size, except with a linear mixed model (*lmer* function), as egg size showed a normal distribution. The successful fertilization rate was

expressed as the percentage of normal embryos relative to total eggs, and the influences of temperature, species and population were tested with a tested with a negative binomial *glmer* with the number of parents crossed as a random factor. The influences of population and species on each subgroup were tested with Kruskal and pairwise Wilcoxon tests.

The influence of the cross, mother, father or temperature on the successful fertilization rate of nursery *A. cytherea* was tested with a negative binomial glmer with the year of testing as a random factor. The significant different fertilization rates of each cross were analyzed with the *dunnTest* function with the Bonferroni adjustment from the *FSA* R package (Ogle et al. 2023). The dissimilarity scores between nursery *A. cytherea* genotypes based on their 20 most abundant symbiont strains was calculated with the Bray Curtis *vegdist* function from the *vegan* R package (Oksanen et al. 2009), then the correlation between dissimilarity and fertilization success for each cross was tested with the cor.test function, at each temperature separately. The multivariate visualization was done with the *PCA* function from the *FactoMine* R package (Lê et al. 2008) and correlations were tested with the *cor.mtest* function from the *corrplot* R package (Wei & Simko 2021). The influence of treatment on *Acropora* recruit densities was tested with Kruskal tests. All test details are reported in Table 4.S1. All statistical analysis was done with RStudio under R version 4.2.1. All scripts and raw data are available on GitHub (github.com/CamiLeonard/Spawning).

# 3. Results

## 3.1 Experiment 1: Spawning pattern of wild versus nursery-reared *Acropora* sp.

### 3.1.1 Spawning pattern

The day of spawning depended on an interaction of species and population (glmer $p < 0.05$, Table 4.S1). In both *A. pulchra* and *A. cytherea*, nursery populations tended to spawn a few days after wild populations, but no differences were noted in other species. In *A. cytherea,* no difference in spawning time between populations was noted. *A. cytherea* spawned precisely 4 h and 9 min ± 2 min (mean ± SEM) after sunset, with wild populations on average 7.4 d after the full moon (AFM) in October and nursery populations on average 9.4 d AFM (Fig. 4.2). *A. cytherea* being studied for three consecutive years, there was a significant interaction of population and year (Table 4.S1): while a delay was observed between nursery and wild populations in 2020 and 2021 (Fig. 4.S3, $p < 0.05$), the populations were synchronized in 2022 ($p = 0.81$). Wild *A. pulchra* populations spawned on average 7.8 d AFM in October and nursery populations on average 10.7 d AFM, approximately 3 h after sunset. *A. hyacinthus* spawned on average 9.4 d AFM in November, approximately 4 h after sunset. *A. nasuta* spawned on average 9.1 d AFM in November, approximately 4 h after sunset. *A. retusa* spawned on average 8.7 d AFM in November, precisely 4 h and 13 min ± 4 min after sunset.

Spawning over more than one night was observed within each of the five species, within 23.1% of genotypes and within 20.3% of individual colonies. *A. hyacinthus* colonies were more likely to spawn over multiple nights than *A. cytherea* or *A. retusa*, and no differences in frequency were observed between wild and nursery populations (Table 4.S1).

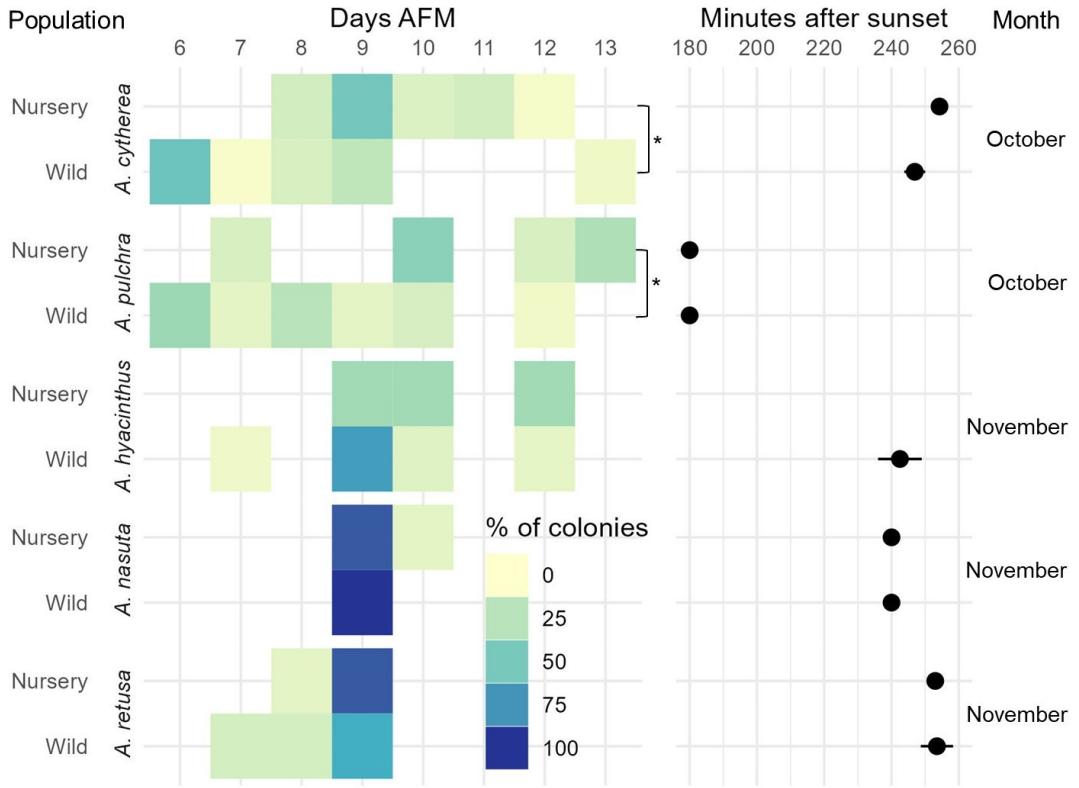



**Figure 4.2.** Spawning patterns of five *Acropora* species in French Polynesia, wild or raised in a nursery, according to the number of days after the full moon, and minutes after sunset with SEM error bars, with * showing significant differences between populations. *A. pulchra* and *A. nasuta* times after sunset are approximative.

### 3.1.2 Fecundity

The fecundity averaged 38.5 ± 2.7 eggs per $cm^2$ of live coral tissue and no significant differences were noted between wild and nurseries populations (Fig. 4.3A, Table 4.S1).

### 3.1.3 Eggs per bundle

The number of eggs per bundle averaged 7.21 ± 0.18 eggs, but it was influenced by the species and the population (Table 4.S1). In *A. retusa* (9.01 ± 0.62 eggs), it was 56.4% higher than *A. hyacinthus* (5.76 ± 0.26 eggs), and 47.7% higher than *A. nasuta* (6.10 ± 0.46 eggs) (Fig. 4.3B). *A. pulchra* (7.41 ± 0.48 eggs) also had 28.6% more eggs per bundle than *A. hyacinthus*. In *A. retusa*, wild colonies had 45.5% larger bundles (10.90 ± 0.69 eggs) than nursery colonies (7.49 ± 0.64 eggs). In other species, the population did not influence the number of eggs per bundle.

### 3.1.4 Egg size

The egg size was significantly influenced by the species (Table 4.S1). *A. hyacinthus* had 7.0% larger eggs (0.646 ± 0.01 mm) than *A. pulchra* (0.604 ± 0.01 mm) and 6% larger than *A. retusa* (0.609 ± 0.01 mm) (Fig. 4.3C). The mean egg size was 0.62 ± 0.004 mm and ranged from 0.53 to 0.73 mm. Population had no significant influence on egg size.

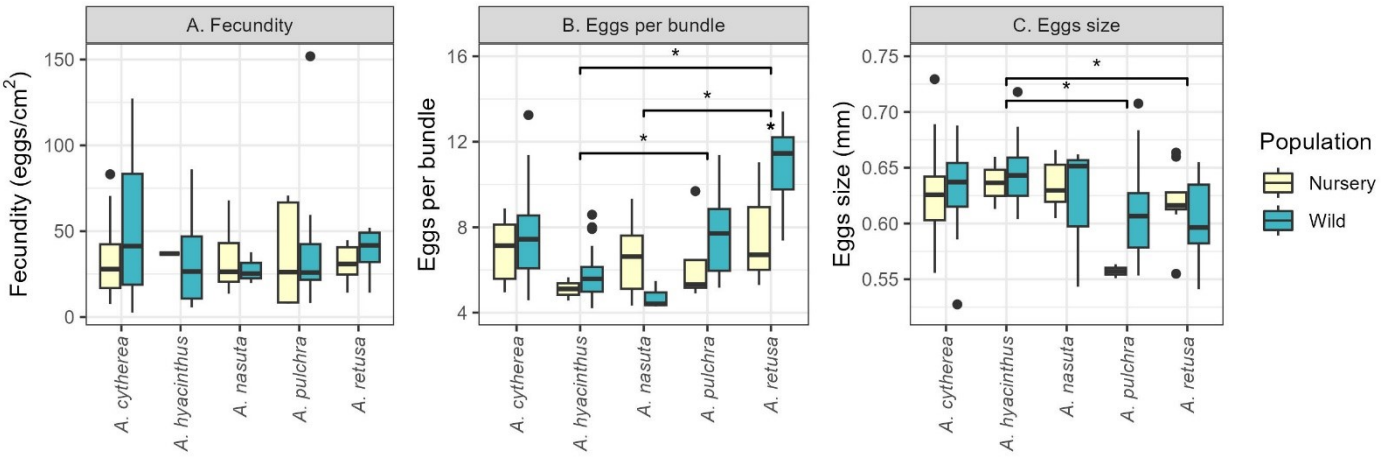



**Figure 4.3.** (A) Fecundity of five species of *Acropora* corals spawning ex situ in Mo'orea, French Polynesia, expressed as number of eggs produced per cm$^2$ of live coral tissue. (B) Number of eggs per bundle of five species of *Acropora*. (C) Egg size (mean diameter) of five *Acropora* species. * indicate significant differences between populations or species (brackets).

### 3.1.5 Fertilization rate

Fertilization rates were influenced by an interaction of temperature, species and population (Table 4.S1). Nursery populations of *A. retusa* had lower fertilization rates than wild populations, both at 27°C (53.6 ± 6.5% in wild vs. 11.9 ± 3.7% in nursery colonies) and 31°C (35.9 ± 5.0% in wild vs. 2.2 ± 0.5% in nursery colonies) (Fig. 4.4). No differences between populations were observed in other species. Fertilization rates at 27°C were different among species, with *A. cytherea* showing the best fertilization rates (72.8 ± 2.6%), higher than *A. nasuta* (61.8 ± 3.9%) and *A. retusa* (32.8 ± 6.0%). *A. nasuta* and *A. hyacinthus* (70.9 ± 4.5%) also had higher fertilization rates than *A. retusa*. At 31°C, *A. hyacinthus* (41.8 ± 4.7%) and *A. nasuta* (45.2 ± 4.8%) both showed higher fertilization rates than *A. cytherea* (21.0 ± 1.8%) and *A. retusa* (19.1 ± 4.6%).

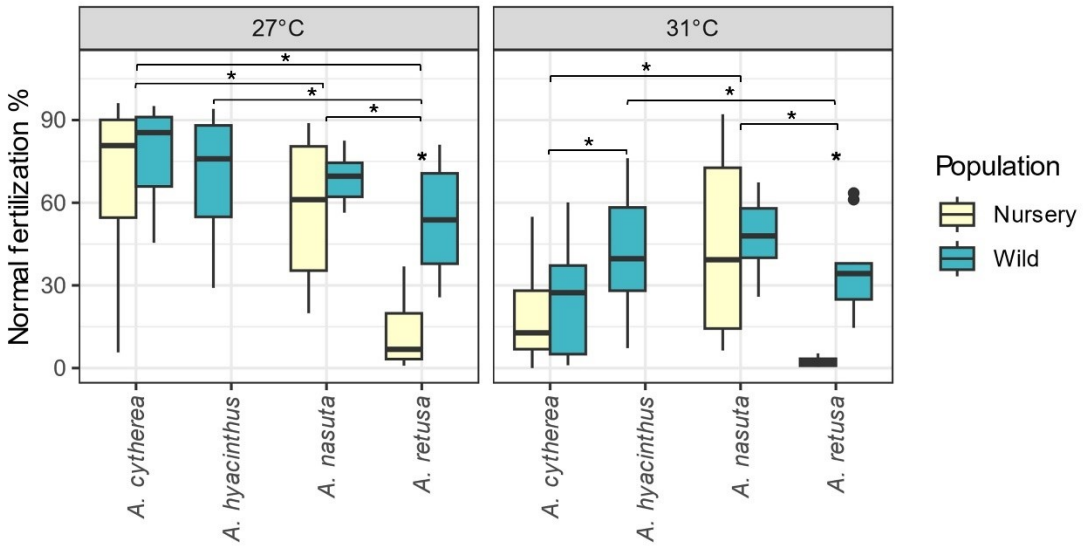



**Figure 4.4.** Fertilization success rates (% of normal embryos) of five species of *Acropora* embryos at 27 and 31°C, from nursery or wild colonies. * represent significantly different species (brackets) or populations.

### 3.1.6 Multivariate analysis

Overall, the species were not distinguished by their reproductive output (Fig. 4.4A). Only the nursery population of *A. retusa* stands out with low fertilization rates (Fig. 4.4A). Notably, the fecundity of corals was significantly positively correlated with the number of eggs per bundle, but eggs per bundle was not significantly correlated with egg size (Fig. 4.4B). The size of the coral was positively linked to its fertilization rate at 27°C. Although the correlation between size and fecundity was not significant ($p = 0.052$), a GLMM showed a significant positive link ($p = 0.002$) between both variables, independent of the species. For all species, the wild colonies had significantly larger sizes than nursery colonies (Table 4.S1).

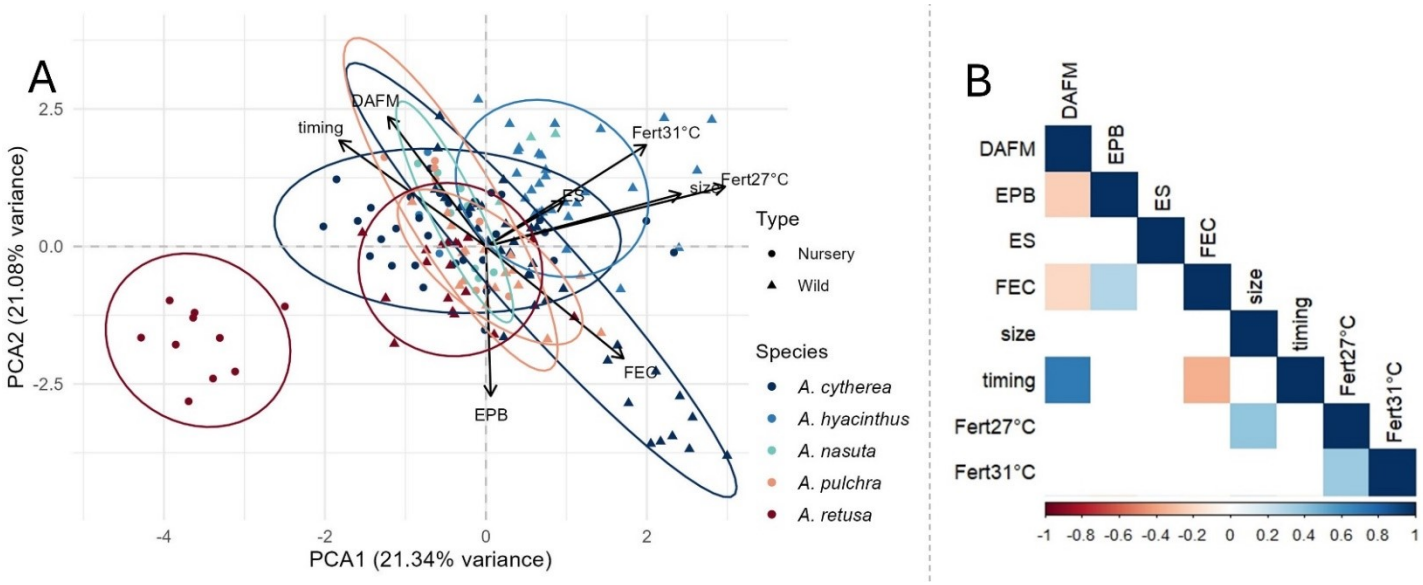



**Figure 4.5.** (A) PCA of reproductive output of five species of *Acropora*, from the wild or nursery-reared. DAFM = days after the full moon, EPB = eggs per bundle, ES = egg size, FEC = fecundity. (B) correlation matrix of reproductive output measures, only correlations with pvalue < 0.05 are shown.

## 3.2 Experiment 2: Nursery *A. cytherea* selective breeding

### 3.2.1 Individual crosses

There were significant differences in successful fertilization rates depending on temperature and on parents crossed (Table 4.S1). At 27°C, 62.5% of the 17 pairs of reciprocal crosses (eggs x sperm and sperm x eggs) had significantly different fertilization successes. At 31°C, 41.2% of reciprocal crosses had significantly different fertilization successes. At both temperatures, the cross, mother and father all significantly influenced fertilization rates (Table 4.S1). At 27°C, the paternal effect was stronger than maternal or the combined effect, while at 31°C, the combined effect was strongest (Table 4.S1). At 27°C, mean fertilization success was 4.9-fold higher than at 31°C (78.0 ± 1.4% versus 15.8 ± 1.0%). At 27°C, fertilization success ranged from 7.3 ± 5.6% to 95.2 ± 0.7%, and at 31°C, it varied from 0.6 ± 0.2% to 55.6 ± 9.5% (91-fold increase) (Fig. 4.6).

At 31°C, the cross 11x2 (sperm x eggs) was the most successful cross (55.6 ± 9.5%), significantly more than the six least successful crosses (1x3, 3x7, 5x3, 8x3, 9x2, 9x3), followed by crosses 7x3 (44.8 ± 3.8%), 2x11 (44.4 ± 2.9%), 11x3 (41.0 ± 2.2%) and 3x11 (40.0 ± 1.4%). In total, nine crosses with significantly higher fertilization success rates were identified (Table 4.S3). The genotype of the mother or the father also influenced fertilization outcome. Eggs from genotype 11 (average 42.2 ± 1.7%) were significantly better than any other genotype except those from genotypes 2 and 8. Eggs from genotypes 1, 2 and 8 were significantly better than those from 3. Sperm from genotype 11 showed the best fertilization rates (average 48.3 ± 5.1%), significantly better than any other sperm except sperm from genotype 7 (21.9 ± 4.4%). Sperm from genotype 3 was also significantly better than sperm from genotype 8 (18.9 ± 2.2% versus 5.5 ± 1.5%).

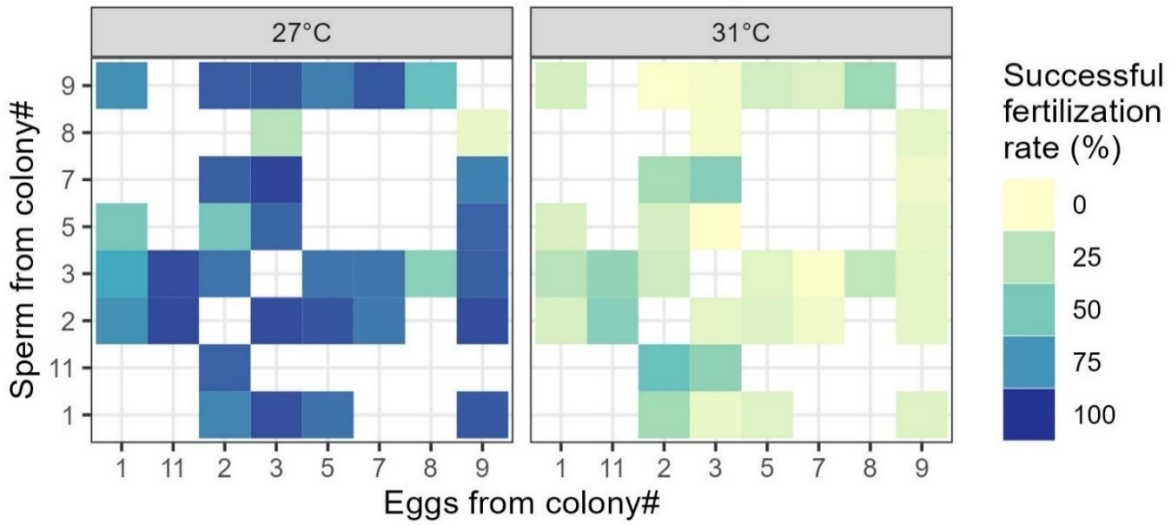



**Figure 4.6.** Fertilization success rates of individual crosses (eight separate genotypes) of nursery-reared *A. cytherea* in Mo'orea, French Polynesia.

### 3.2.2 Influence of symbiont assemblages

Genotypes of nursery *A. cytherea* could be discriminated based on both their reproductive output and symbiont communities (Fig. 4.7A). While genotypes 5, 8 and 9 harbored a majority of *Durusdinium*, genotypes 1, 3 and 7 had a majority of *Cladocopium* and genotype 2 an equivalent mix of *Symbiodinium* and *Cladocopium*. The abundance of *Symbiodinium* in nursery *A. cytherea* was positively correlated to the number of eggs per bundle and to the fertilization success of eggs at 31°C (Fig. 4.7B). The abundance of *Cladocopium* was positively correlated to the fertilization success of eggs at 27°C and sperm at both temperatures (Fig. 4.S4). The abundance of *Durusdinium* was negatively correlated to the fertilization success of eggs at 27°C and sperm at both temperatures (Fig. 4.S4). Unlike the size for all species combined, the size of nursery *A. cytherea* was not correlated with any other measure. Because of too few replicates, no genotype was significantly better than another in ES, EPB or fecundity, but visually, genotypes 11, 3 and 7 were characterized by high fertilization rates and fecundity and genotype 2 by high egg fertilization rates at 31°C and EPB (Fig. 4.7A).

The fertilization success rates at 31°C were strongly negatively correlated to the dissimilarity scores of the symbiont communities of the genotypes: the genotypes with similar symbiont communities had thus also better fertilization rates at 31°C. At 27°C, no significant correlations were observed (Table 4.S1). Omitting genotype 11 because its symbiont diversity was not analyzed, the best crosses at 31°C were 1x2 and 7x2, and the worst crosses were 5x3, 9x2 and 9x3. 1 and 2 had a 0.53 dissimilarity score, 2 and 7 0.51, 5 and 3 0.98, 2 and 9 0.96, and 3 and 9 0.98.

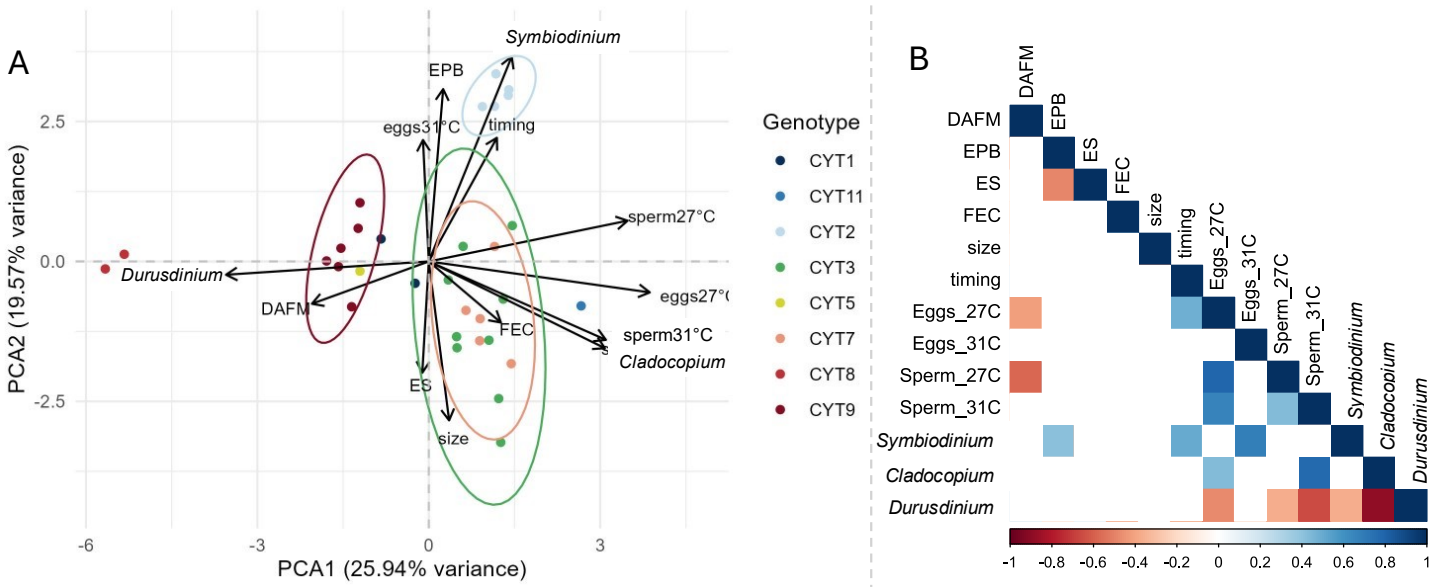



**Figure 4.7.** (A) PCA of reproductive output of all nursery *A. cytherea* and the average abundance of each symbiont genus in their genotype. DAFM = days after the full moon, EPB = eggs per bundle, ES = egg size, FEC = fecundity. (B) Correlation matrix between reproductive output of all nursery *A. cytherea* and the average abundance of each symbiont genus in their genotype. Only correlations with pvalue < 0.05 are shown.

## 3.3 Experiment 3: Larval seeding

Ten days after larval seeding, the coral recruit density on the top of plastic settlement plugs was 10.9 ± 3.5 recruits (mean ± SEM) per 100 $cm^2$ on seeded bommies, significantly more than on control plugs, as no recruits were observed on control plugs (Fig. 4.8A). After one year, all *Acropora* recruits were counted on all bommies, and no new *Acropora* recruits were noted on any of the control bommies. On the seeded bommies, the *A. cytherea* recruits were visible with the naked eye. Their density on the seeded bommies averaged 0.11 ± 0.04 recruits per 100 $cm^2$, dropping by a factor of 100 after one year (Fig. 4.8B). The size of the seeded recruits was 2.7 ± 0.3 $cm^2$ after 16 months and 6.1 ± 0.7 $cm^2$ after 21 months.

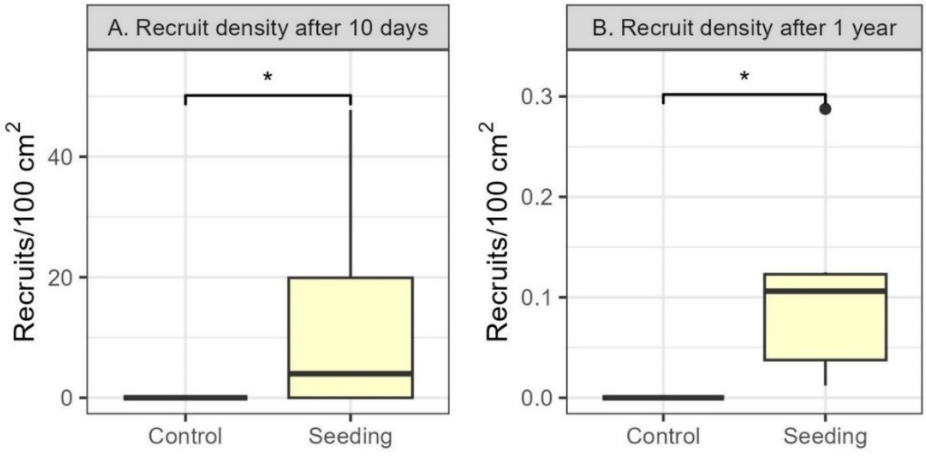



**Figure 4.8.** (A) Recruit density on pre-conditioned plastic settlement plugs, ten days after larval seeding within mesh nets in situ. (B) *Acropora* recruit density on whole bommies, one year after larval seeding within mesh nets in situ.

## 4. Discussion

As coral nurseries become more widespread, it is important to consider the potential effects of a modified environment or a modified growth through suspension on the health and reproduction of coral colonies. Sexual reproduction is a key aspect of the coral's life cycle for the maintenance of genetic diversity. In fact, if growing coral clones in nurseries allowed to have a fast growth but a low reproductive rate, the resulting corals used for restoration practices would risk having low genetic diversity and not efficiently enhance the larval supply. The present study shed light on spawning timing, fecundity, egg size, and fertilization rates for five species of *Acropora* and compared wild populations to nursery-reared ones. Nursery rearing proved to yield corals with reproductive patterns and outputs comparable to wild populations. A case study on *A. cytherea* on the potential of restoration using sexual reproduction demonstrated that a promising way to improve fertilization rates was to specifically select certain crosses that yield higher quality embryos than others, especially at elevated temperatures. Furthermore, the extremely low settlement rate observed in the lagoon was durably enhanced by assisted settlement of larvae within nets.

### Timing

We found no delay in spawning day and time between nursery and wild populations of *A. hyacinthus*, *A. nasuta* and *A. retusa*, but an apparent delay in the day of spawning for nursery *A. pulchra* and *A. cytherea* populations. However, this was dependent on the year of sampling, as *A. cytherea* populations were synchronized during the third spawning event. *A. pulchra* might follow the same tendency but was only monitored for one spawning event. Potentially, *A. cytherea* and *A. pulchra,* which are typically found in very shallow reefs (Wallace 1999), could show an initial response to being moved to a deeper nursery (5 m), as transplantation to a lower maximum temperature or light intensity could explain a slight delay in the day of spawning (Paxton et al. 2016; Davies et al. 2023). These species could then synchronize again with wild populations after a few years. Similarly, in *A. cervicornis* reared for 1-2 years in suspended nurseries, spawning falls within the expected window for that species, although no direct comparison of wild versus nursery colonies were conducted (Koch et al. 2022b). Split spawning, that is spawning over consecutive nights for the same colony, can happen when different areas of a colony are shaded which can delay the maturation of gametes (Shimoike et al. 1993); we observed it for 20% of all colonies, with no difference in occurrence between wild and nursery-reared ones. As the lagoon nursery benefits from the same environmental cues as wild populations for the onset of spawning time, we did not expect large variations in spawning day or time between populations. The overall synchrony with wild populations was maintained in nursery populations.

### Reproductive output

The reproductive output, characterized by egg size, eggs per bundle (EPB), fecundity (eggs per $cm^2$) and fertilization rates at 27 and 31°C did not vary between wild and nursery populations. The only exception was for nursery *A. retusa*, which had less EPB and a lower fertilization rate

than wild colonies, despite a similar egg size and fecundity. This species presents thus a distinct pattern from others. As we saw positive links between colony sizes and fertilization rates, similarly to observations in other studies (Koch et al. 2022a), the slow growth rate of *A. retusa*, with its thicker branches (Kahng et al. 2024), relative to other species (see chapter 2), might not allow them to reach the same reproductive output as the other species. Alternatively, *A. retusa* from the nursery produced gametes with reduced quality or showed lower fertilization compatibility than wild populations for yet undetermined reasons, which could be the focus of future experiments. For the other four studied species, nursery rearing did not lead to a modified reproductive output.

The reproductive output was more influenced by the species than the population, and was comparable to observations from previous studies on *Acropora* sp. (Wallace 1985; Okubo et al. 2007; Carroll 2009; Foster & Gilmour 2020). While egg size can increase fertilization rates (Levitan 1993) and be negatively correlated to EPB (Hall & Hughes 1996; Padilla-Gamiño & Gates 2012), our data did not corroborate such trend. Other authors also revealed no apparent link between egg size and fertilization rates in *Acropora* (Foster & Gilmour 2020). Fertilization rates were strongly reduced at high temperatures, corroborating previous observations (Negri et al. 2007; Albright & Mason 2013; Humanes et al. 2017). This can be due to a reduction of sperm motility or the deformation of eggs (Negri et al. 2007; Randall & Szmant 2009; Keshavmurthy et al. 2014). *A. hyacinthus* and *A. nasuta* showed the best fertilization rates at 31°C, so these species should be targeted for future restoration efforts, as they can produce offspring fit to resist extreme heat stress events.

## Selective breeding

While coral fertilization success is generally tested on a pool of parents, the role of individual parents or separate sexes is less frequently evaluated. Within the same population of nursery *A. cytherea*, we observed a very large variability in fertilization rates depending on temperature and different individual crosses. Fertilization could be improved 13-fold at 27°C and up to 91-fold at 31°C when pairing the right parents. This corroborates with previous studies demonstrating genotype-specific gamete quality and varying gamete compatibility (Baums et al. 2013; Miller et al. 2018). Gamete compatibility is mediated by sperm chemotaxis through chemical signals released by eggs and species-specific gamete recognition proteins (GRPs), and sperm can preferentially swim towards the most compatible eggs (Evans & Sherman 2013). A possible explanation for varying fertilization rates is the existence of different morphs that lack compatibility (Willis et al. 1997). Also, differences in gamete morphology or gamete age can constitute prezygotic barriers preventing fertilization (Levitan et al. 2004; Baums et al. 2013). In our case, *A. cytherea* colonies were morphologically similar and spawned at the same time thus gametes had the same age, but gamete morphology was not studied which could have impacted fertilization success. The same two parents mostly had different fertilization successes when using either their eggs or sperm (reciprocal crosses). This was not observed in *A. cervicornis* individual crosses, where all reciprocal crosses showed the

same fertilization success, although fertilization success varied between different crosses (Koch et al. 2022b).

## Influence of symbionts

While we did not study host genetics, we observed a correlation between symbiont diversity and parent compatibility for the heat treatment (31°C), with parents having more similar symbionts also showing better compatibility. This might just be linked to the genotype compatibility, as genotype strongly influences the microbiome composition in *Acropora* (Baums et al. 2014; Aguirre et al. 2022). In *Montipora*, who transmits symbionts to their descendants, symbiont identity also did not influence parent compatibility at ambient temperature (Johnston et al. 2024). To our knowledge, no other study observed links between symbiont diversity and gamete compatibility, but the symbiont community can influence gene expression in adult *Acropora* (Barfield et al. 2018) and in their coral larvae after symbiont acquisition (Buerger et al. 2020), as well as the survival of juveniles (Quigley et al. 2016). At ambient temperature, 8-day-old *Montipora* larvae from parents with different symbiont communities showed no differences in survivorship, but at high temperatures, larvae from a mix of all parents had the best survivorship, followed by larvae from heat-resistant parents containing mostly *Durusdinium* sp., while larvae from heat-sensitive parents, containing mostly *Cladocopium* sp, had the lowest survivorship (Drury et al. 2021). In another study on *Montipora,* there were strong maternal effects linked to larval heat tolerance, but none were linked to the parent's symbiont identity despite a vertical transmission (Johnston et al. 2024).

We found strong paternal, maternal, and combined effects on fertilization success at both ambient and elevated temperature. Other experiments also found strong combined and maternal effects on larval survival at high temperatures (Dixon et al. 2015; Johnston et al. 2024). The improved fertilization rate at 31°C was also positively linked to the abundance of *Symbiodinium* sp. and *Cladocopium* sp., but negatively with *Durusdinium* sp.. Curiously, *Durusdinium* sp. is usually associated with heat-resistant adult corals (Berkelmans & Van Oppen 2006), but trade-offs towards lower energetic reserves and a production of smaller eggs have been observed (Jones & Berkelmans 2011), although we observed no link between egg size and symbiont communities. In *Acropora*, as there is no vertical transmission of symbionts, a heat tolerance though a dominance of *Durusdinium* in adults could hide a trade-off to a lower quality of gametes produced (Jones & Berkelmans 2011), thus explaining the lower fertilization rate observed. A lower fertilization rate is, however, not synonymous with a lower heat resistance in juveniles. Even if symbionts are not transmitted vertically in *Acropora*, mitochondrial proteins are inherited from the mother colony and can cause an inherited thermal tolerance in the offspring (Dixon et al. 2015). Further experiments should determine whether an improved fertilization at high temperature is linked to larval thermal resistance.

## Larval seeding

Once the best parents have been selected for larval production, the major challenges to overcome are the massive larval and recruit mortality observed in situ (Martinez & Abelson

2013; Miller et al. 2020). These could be strongly reduced by protecting larvae in tanks or in floating enclosures (Suzuki et al. 2020), then enhancing their settlement success on appropriate substrate. Since the late 1990's, a handful of projects have demonstrated that larval seeding could accelerate reef recovery (Heyward et al. 2002; Omori et al. 2003; dela Cruz & Harrison 2017; Harrison et al. 2021). Similarly to their results, we found that a simple weighted net system proved to be highly effective for concentrating larval densities on a degraded site and for significantly improving recruitment in this area. Although initial mortality was very high, *A. cytherea* recruits were still present on seeded bommies after 21 months. *A. cytherea* can be an efficient species in coral restorations as it shows very fast growth rates (Clark & Edwards 1995). Success of a seeding trial can strongly depend on the chosen site (Randall et al. 2023). For instance, in areas where natural recruitment is not impaired, larval seeding will not have any long-term benefits (Edwards et al. 2015). Here, we found no natural *Acropora* recruitment after the seeding on any of the monitored bommies. The larger the nets, and the more larvae injected into them, the more cost-effective the seeding project can become, yielding hundreds of adult colonies after three years for around 18USD per colony (dela Cruz & Harrison 2020; Harrison et al. 2021). The larval seeding technique could thus be a practical way to restore damaged sites, likely more cost-effective than direct transplantation of adult colonies (Doropoulos et al. 2019a).

Previous larval seeding efforts often used complex setups with large quantities of specific materials (Heyward et al. 2002; Omori et al. 2003; dela Cruz & Harrison 2017). We wanted to provide a proof of concept of this restoration technique but using less important and specific means. We therefore designed larval nets with easily available materials (thin fabric, chain, and bicycle tubes), that we filled with larvae with the help of common tools (large water bottles, funnel and hose), without needing scuba diving equipment. We found promising results, with recruits surviving at least 21 months following seeding, compared to zero natural *Acropora* recruits on any control bommy. A larval seeding protocol with reduced means and investments could be a valuable way to involve local communities that lack financial aids in their efforts to restore coral reefs. In fact, a lack of community involvement is often cited as a significant limitation in coral restoration projects (Hein et al. 2019). As the dispersal distance of Acroporid corals might be much lower than previously expected (Shinzato et al. 2015; Zayasu et al. 2016), assisted seeding may become necessary in the future to restore some isolated reefs that suffered heavy *Acropora* mortality following a bleaching event or crown-of-thorns outbreaks for instance.

## Further considerations and recommendations for managers

Corals aimed at restoration projects and grown in nurseries should be ensured to maintain a natural reproductive cycle. In fact, outplanted corals should integrate their new population by adding to the genetic diversity, which necessitates a synchrony of their spawning patterns with those of wild populations. Corals could also be specifically chosen for their increased resistance to predicted stress events. We found *A. hyacinthus* and *A. nasuta* to have better fertilization rates at elevated temperatures than other species, with the same fecundity and a

highly synchronized day and time of spawning, despite two years of nursery rearing. *A. nasuta* has also previously been shown to display a fast growth rate and a lower size necessary to reach maturity than other *Acropora* species (Chapter 2), thus this species could thus be a promising candidate in future restoration efforts.

Considering the highly improved fertilization rates at elevated temperatures when selecting compatible parents, a quick series of selective crosses could help determine which corals to prioritize for restoration projects. In Mo'orea, crosses of *A. cytherea* nursery genotypes 11 and 2, 11 and 3 and 7 and 3 produced the most resistant embryos at elevated temperatures. Curiously, these were the genotypes with the lowest abundance of heat-resistant symbiont *Durusdinium* sp., indicating a potential trade-off in reproductive output and that resistant parents do not necessarily produce the most resistant offspring. The identified nursery genotypes should be further studied for their promising capacity to produce more resistant offspring at elevated temperatures, and for determining whether parental effects or the onset of symbiosis in recruits impacts their future heat tolerance. These genotypes could serve to produce large quantities of larvae to seed damaged sites. A strong genotype specificity in fertilization success might lead to an important reduction in recruitment if the genotype richness decreases on the reef: thus it is important to protect genotypic richness as well as total population size in restoration efforts (Baums et al. 2013).

A priority should be to identify sites most needing recovery, for instance lagoon sites with low diversity and extremely low natural recruitment, such as we observed in this study. Other sites to restore are those that have suffered mass bleaching events or crown-of-thorn outbreaks for instance. We demonstrated that larval seeding can be successful even with common materials and reduced means. Larval seeding efforts could even become automated on degraded reefs thanks to AUVs like the LarvalBot (Dunbabin et al. 2020), or be further improved by increasing the availability of protection on the denuded reef substrate: either by adding holes through drilling (Nozawa 2008) or adding complex artificial materials with micro-refuges to the substrate (Suzuki et al. 2011; Randall et al. 2021), which would help optimize recruit growth and survival (Vardi et al. 2021). To summarize, combining different restoration techniques for a more holistic approach to the restoration process could significantly improve its outcomes.

## Conclusion

A better understanding of the parameters influencing the sexual reproduction of corals, the impact of nursery rearing on coral health and the long-term results of restoration practices is crucial if we want restoration methods to be efficient, replicable, and scalable. We demonstrated that nursery rearing over more than two years yielded corals with a reproductive output as good as that of wild colonies. We also found that when specific pairs of corals were selected, they can yield a significantly improved fertilization, even under temperature stress. Those genotypes could potentially be used to produce heat-resistant descendants in the future. A low-effort method to significantly increase recruitment rate in degraded areas was the seeding of coral larvae within mesh nets, removed after two days. The

present results will hopefully contribute to fine-tuning restoration efforts and improve their outcomes in the future.

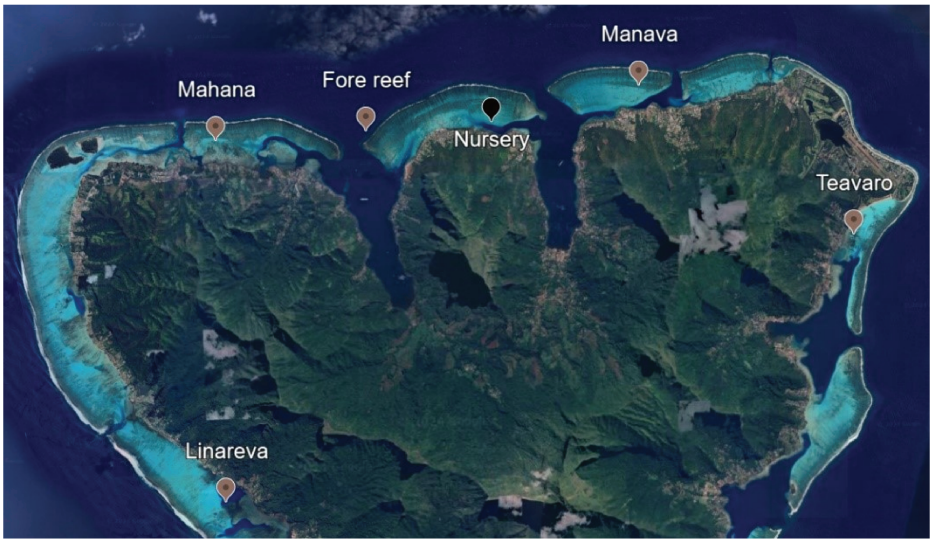
## Acknowledgements

As guests, we recognize and give thanks for the land and water resources of Mo'orea, and to the traditional owners of the land. We would like to acknowledge all the CRIOBE staff and students who helped during the data collection, especially Hugo Bischoff, Alex Fellous, Mathieu Reynaud, Camille Vizon, Chloé Pozas, Cyrielle Rigal, Isa Davezies, Claire Boitel, Jules Schligler, Guillaume Iwankow and Camille Gache. This research was funded by the AFR Grant from the FNR Luxembourg.

# 5. Supplementary materials

Table 4.S1: Statistical tests of different experiments

| Experiment | Test | Subset | Response variable | Predictor | $X^2$ | df | p |
|---|---|---|---|---|---|---|---|
| **Timing** | glmer (negbinomial, | All | **Days after full moon** | **Species** | **29.56** | **4** | **<0.001** |
| | genotype as | | | **Population (Wild vs Nursery)** | **29.14** | **1** | **<0.001** |
| | random factor) | | | **Species*Population** | **11.54** | **4** | **0.021** |
| | glmer (negbinomial, | *A. cytherea* | **Days after full moon** | **Population (Wild vs Nursery)** | **29.84** | **1** | **<0.001** |
| | genotype as | | | **Year** | **19.37** | **2** | **<0.001** |
| | random factor) | | | **Population*Year** | **29.46** | **2** | **<0.001** |
| | Kruskal | *A. pulchra* | **Days after full moon** | **Population (Wild vs Nursery)** | **7.94** | **1** | **0.004** |
| | Kruskal | *A. hyacinthus* | Days after full moon | Population (Wild vs Nursery) | 2.85 | 1 | 0.091 |
| | Kruskal | *A. nasuta* | Days after full moon | Population (Wild vs Nursery) | 0.3 | 1 | 0.584 |
| | Kruskal | *A. retusa* | Days after full moon | Population (Wild vs Nursery) | 1.82 | 1 | 0.178 |
| | Kruskal | *cytherea* | Minutes after sunset | Population (Wild vs Nursery) | 1.39 | 1 | 0.238 |
| | glmer (binomial, | all | Split spawning | **Species** | **11.17** | **4** | **0.025** |
| | genotype as | | | Population (Wild vs Nursery) | 1.18 | 1 | 0.277 |
| | random factor) | | | Species*Population | 0.95 | 4 | 0.918 |
| | Pairwise Wilcoxon | All species | Split spawning | ***A. hyacinthus vs A. cytherea*** | | | **0.009** |
| | | | | ***A. hyacinthus vs A. retusa*** | | | **0.037** |
| **Fecundity** | glmer (negbinomial, | All | Fecundity | Species | 4.98 | 4 | 0.29 |
| | genotype as | | | Population (Wild vs Nursery) | 0.74 | 1 | 0.388 |
| | random factor) | | | Species*Population | 1.04 | 4 | 0.904 |
| | glmer (negbinomial, | Measured sizes | **Fecundity** | **Coral size** | **9.52** | **1** | **0.002** |
| | genotype as | | | Species | 5.71 | 3 | 0.127 |
| | random factor) | | | Coral size*Species | 0.92 | 3 | 0.82 |
| **Eggs per bundle** | glmer (negbinomial, | All | Eggs per bundle | **Species** | **49.51** | **4** | **<0.001** |
| | genotype as | | | **Population (Wild vs Nursery)** | **7.22** | **1** | **0.007** |
| | random factor) | | | **Species*Population** | **11.52** | **4** | **0.021** |
| **Egg size** | lmer(genotype as | All | Egg size | **Species** | **15.45** | **4** | **0.004** |
| | random factor) | | | Population (Wild vs Nursery) | 0.33 | 1 | 0.563 |
| | | | | Species*Population | 5.08 | 4 | 0.279 |
| **Fertilization** | glmer (negbinomial, | All | Normal fertilization | **Temperature** | **296.98** | **1** | **<0.001** |
| | number of parents | | | **Species** | **174.95** | **3** | **<0.001** |
| | as random factor) | | | **Population (Wild vs Nursery)** | **10.15** | **1** | **0.001** |
| | | | | **Temperature*Species** | **51.32** | **3** | **<0.001** |
| | | | | Temperature*Population | 0.21 | 1 | 0.645 |
| | | | | **Species*Population** | **76.06** | **2** | **<0.001** |
| | | | | **Temperature*Species*Popula** | **13.86** | **2** | **<0.001** |
| **Size** | glmer (negbinomial, | All | Colony size | **Population** | **21.95** | **1** | **<0.001** |
| | genotype as | | | **Species** | **34.04** | **3** | **<0.001** |
| | random factor) | | | Population*Species | 2.59 | 2 | 0.274 |
| **Individual crosses** | glmer (negbinomial, | All | Normal fertilization | **Temperature** | **1455.15** | **1** | **<0.001** |
| | year as random | | | **Cross** | **268.42** | **33** | **<0.001** |
| | factor) | | | **Temperature*Cross** | **378.66** | **32** | **<0.001** |
| | glmer (negbinomial, | 27°C | Normal fertilization | **Cross** | **40.63** | **18** | **0.002** |
| | year as random | | | **Female** | **43.31** | **7** | **<0.001** |
| | factor) | | | **Male** | **144.16** | **7** | **<0.001** |
| | glmer (negbinomial, | 31°C | Normal fertilization | **Cross** | **141.87** | **19** | **<0.001** |
| | year as random | | | **Female** | **54.2** | **7** | **<0.001** |
| | factor) | | | **Male** | **26.94** | **7** | **<0.001** |
| | cor.test | 27°C | Normal fertilization | Dissimilarity in symbionts | 0.109 | 28 | 0.566 |
| | | **31°C** | **Normal fertilization** | **Dissimilarity in symbionts** | **-0.378** | **28** | **0.04** |
| **Larval seeding** | Kruskal | All | 10-day recruit density | **Treatment** | **13.89** | **1** | **<0.001** |
| | Kruskal | All | 12-months recruit der | **Treatment** | **9.47** | **1** | **0.002** |




| Site | GPS coordinates |
|---|---|
| Nursery | 17°29'01.1"S 149°50'03.9"W |
| Linareva | 17°33'13.4"S 149°53'08.5"W |
| Mahana | 17°29'13.6"S 149°53'15.6"W |
| Manava | 17°28'36.7"S 149°48'21.4"W |
| Fore reef | 17°29'07.3"S 149°51'31.1"W |
| Teavaro | 17°30'15.4"S 149°45'51.7"W |

**Figure 4.S1.** Map of sampling sites and coral nursery location

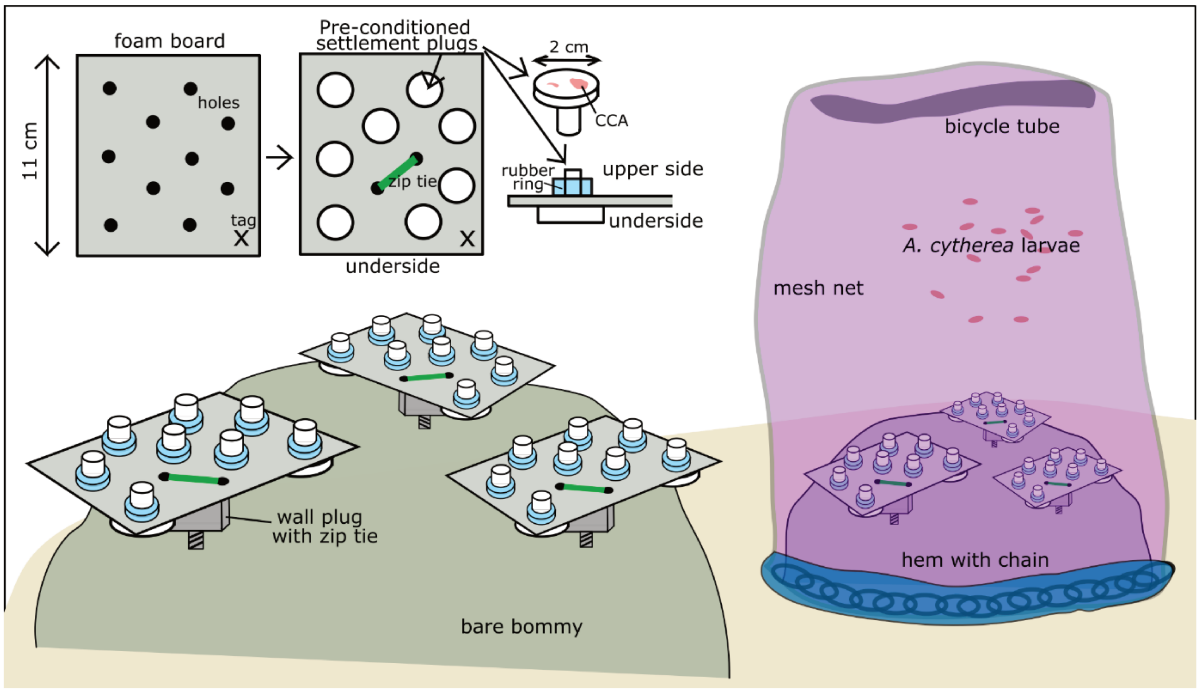



**Figure 4.S2.** Experimental setup of settlement tiles on seeded and control bommies in the lagoon of Mo'orea, French Polynesia.

**Table 4.S2.** Number of *Acropora* colonies which spawned and were used for the described experiments

| | Year | Nursery | Wild |
|---|---|---|---|
| *A. pulchra* | 2020 | 4 | 15 |
| *A. hyacinthus* | 2020 | 2 | 20 |
| *A. nasuta* | 2020 | 9 | 3 |
| *A. retusa* | 2020 | 8 | 14 |
| *A. cytherea* | 2020 | 8 | 12 |
| | 2021 | 7 | 10 |
| | 2022 | 10 | 10 |

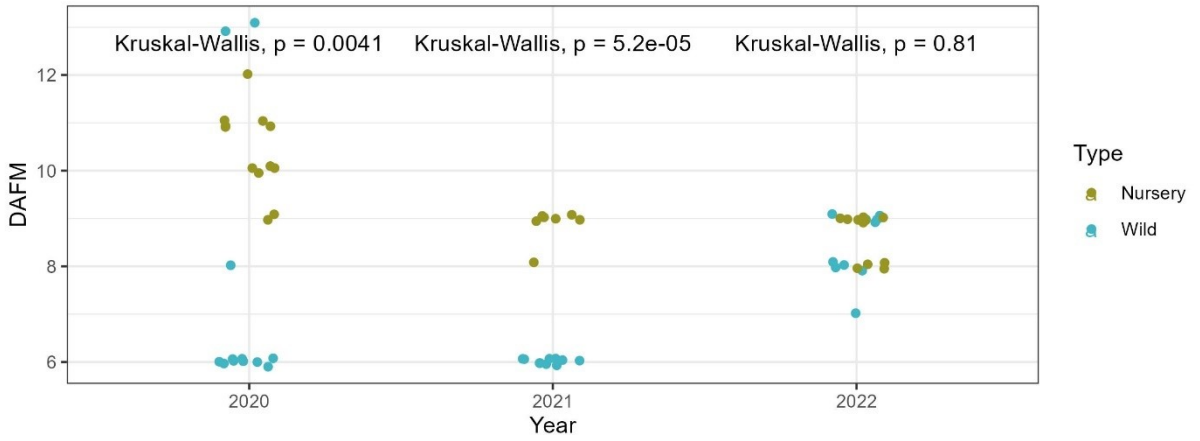



**Figure 4.S3.** Timing of *A. cytherea* spawning (Days after the full moon) each year, in nursery and wild populations.

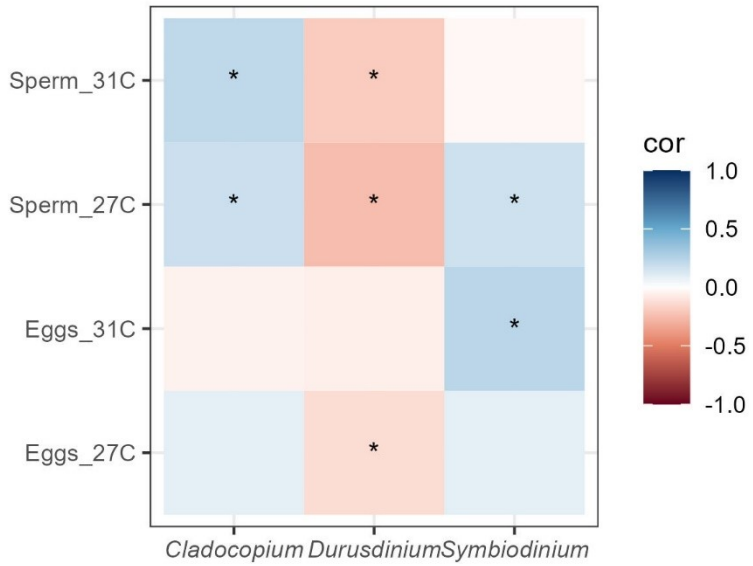



**Figure 4.S4.** Correlation plot based on all individual crosses of nursery *A. cytherea* at 27 and 31°C, with symbiont genus averaged for each genotype.

**Table 4.S3.** Dunn pairwise comparisons of fertilization success rates between each cross of *A. cytherea* genotypes, at 31°C

| Comparison | Z | P.unadj | P.adj |
|---|---|---|---|
| 11x2 - 1x3 | 4.00 | <0.001 | 0.036 |
| 11x2 - 3x7 | 4.15 | <0.001 | 0.019 |
| 11x2 - 5x3 | 5.41 | <0.001 | <0.001 |
| 11x2 - 8x3 | 4.40 | <0.001 | 0.006 |
| 11x2 - 9x2 | 4.32 | <0.001 | 0.009 |
| 11x2 - 9x3 | 4.43 | <0.001 | 0.005 |
| 11x3 - 3x7 | 4.01 | <0.001 | 0.034 |
| 11x3 - 5x3 | 5.25 | <0.001 | <0.001 |
| 11x3 - 8x3 | 4.25 | <0.001 | 0.012 |
| 11x3 - 9x2 | 4.19 | <0.001 | 0.016 |
| 11x3 - 9x3 | 4.28 | <0.001 | 0.011 |
| 1x2 - 1x3 | 4.25 | <0.001 | 0.012 |
| 1x2 - 3x7 | 4.18 | <0.001 | 0.017 |
| 1x2 - 5x3 | 5.81 | <0.001 | <0.001 |
| 1x2 - 8x3 | 4.61 | <0.001 | 0.002 |
| 1x2 - 9x2 | 4.37 | <0.001 | 0.007 |
| 1x2 - 9x3 | 4.65 | <0.001 | 0.002 |
| 1x3 - 2x11 | -3.96 | <0.001 | 0.043 |
| 1x3 - 7x3 | -3.95 | <0.001 | 0.045 |
| 2x11 - 3x7 | 4.11 | <0.001 | 0.022 |
| 2x11 - 5x3 | 5.37 | <0.001 | <0.001 |
| 2x11 - 8x3 | 4.36 | <0.001 | 0.007 |
| 2x11 - 9x2 | 4.29 | <0.001 | 0.010 |
| 2x11 - 9x3 | 4.39 | <0.001 | 0.006 |
| 3x1 - 5x3 | 4.82 | <0.001 | 0.001 |
| 3x11 - 3x7 | 3.99 | <0.001 | 0.038 |
| 3x11 - 5x3 | 5.22 | <0.001 | <0.001 |
| 3x11 - 8x3 | 4.22 | <0.001 | 0.014 |
| 3x11 - 9x2 | 4.16 | <0.001 | 0.018 |
| 3x11 - 9x3 | 4.25 | <0.001 | 0.012 |
| 3x7 - 7x3 | -4.11 | <0.001 | 0.023 |
| 3x8 - 5x3 | 4.55 | <0.001 | 0.003 |
| 5x3 - 7x2 | -4.06 | <0.001 | 0.028 |
| 5x3 - 7x3 | -5.36 | <0.001 | <0.001 |
| 7x3 - 8x3 | 4.36 | <0.001 | 0.007 |
| 7x3 - 9x2 | 4.28 | <0.001 | 0.011 |
| 7x3 - 9x3 | 4.38 | <0.001 | 0.007 |

# Chapter 5

## Performance of innovative materials as recruitment substrates for coral restoration

# Performance of innovative materials as recruitment substrates for coral restoration

**Running head:** Innovative materials for coral restoration

*Camille Leonard[1], Laetitia Hédouin[1,2], Margaux C. Lacorne[1,3], Julien Dalle[4], Matthieu Lapinski[4], Philippe Blanc[5], Maggy M. Nugues[2,6]*

[1] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, BP 1013, 98729 Papetoai, Mo'orea, French Polynesia

[2] Laboratoire d'Excellence "CORAIL", Papetoai, Mo'orea, French Polynesia

[3] Université des Antilles, Fouillole, Pointe-à-Pitre 97157, Guadeloupe

[4] Seaboost, 889 rue de la Vieille Poste 34000 Montpellier, France

[5] TotalEnergies, Avenue Larribau, 64018 Pau Cedex, France

[6] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan, 52 Avenue Paul Alduy, 66860 Perpignan Cedex, France

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**Author contributions:** LH, JD, ML, MN conceived and designed the research; CL, MCL, LH performed the experiments; CL, MN analyzed the data; LH, JD, ML, PB contributed reagents/materials/analysis tools; CL, MN wrote the initial draft; all authors edited the manuscript.

## Abstract

Artificial reefs and, more recently, ecoengineering, are frequently advocated as possible tools to counteract the loss of tropical coral reefs worldwide. Despite increasing availability of novel materials, there is limited understanding of how different materials, their physical and chemical properties can influence coral recruitment success and early benthic community development. This study investigated the efficacy of several innovative materials as recruitment substrates for corals and other sessile benthic communities. Stacks of vertically oriented tiles made of eight innovative materials and two common (control) materials were deployed for six months during major coral spawning events on the forereef of Mo'orea, French Polynesia. Tiles were separated from their neighbors by 15 mm to mimic cryptic habitats that are sheltered from predation and typically favored by coral recruits. Six innovative materials, including 3D printed concrete, PVC with chitosan coating, fiberglass polymer and flax-based polylactic acid, produced similar coral recruitment to control materials (Portland concrete and PVC). Two materials (porous concrete and ceramic foam) produced lower

recruitment. Porous concrete was characterized by a high abundance of non-coralline encrusting red algae, which negatively correlated with coral recruitment, while ceramic foam was prone to erosion. The results suggest the structural micro-complexity and durability of an artificial material and the composition of the benthic communities colonizing it can strongly influence coral recruitment. This study highlights several innovative materials as suitable recruitment substrates for coral restoration and provides a better understanding of the properties of artificial materials that are critical for coral recruitment success.

**Key words** Artificial reef • Algae • Competition • 3D printing • *Pocillopora* • Settlement • Benthos • South Pacific

## Implications for Practice

- Innovative materials such as 3D printed concrete, PVC with chitosan coating, fiberglass polymer and flax-based polylactic acid are promising materials for use as coral recruitment substrates on artificial reefs.
- Materials that (1) are durable and (2) harbor micro-grooves effectively support high coral recruitment.
- Materials encouraging the recruitment of non-coralline encrusting red algae deter coral recruitment, while materials colonized by thin turf algae (less than 5 mm in height) and encrusting foraminiferans support high coral recruitment.

# 1. Introduction

Tropical coral reefs are being degraded worldwide due to combinations of various anthropogenic stressors (Hughes et al. 2017a). In response to this degradation, there is a growing need to strengthen the resilience of coral reefs to cope with disturbances. Coral reef resilience can be enhanced using 'passive' or 'proactive' methods of human intervention (e.g., implementation of marine reserves, regulation of fisheries) and 'active' or 'reactive' measures whereby humans directly manipulate the dynamics of degraded reef systems (e.g., coral propagation, artificial reefs, ecoengineering) (Rinkevich 2008; Boström-Einarsson et al. 2020a; Hein et al. 2020; Airoldi et al. 2021). Active restoration techniques typically involve labor-intensive and small-scale transplantation of coral fragments raised in nurseries (known as "coral gardening") (Shafir et al. 2006b). Yet, in the face of global climate change, large-scale restoration techniques, such as 'mass seeding' approaches using wild (Doropoulos et al. 2019a; Suzuki et al. 2020) and laboratory raised coral larvae (Edwards et al. 2015; dela Cruz & Harrison 2017; Chamberland et al. 2017), are increasingly being considered to complement proactive management interventions.

Artificial reefs are a popular approach for active coral restoration (Clark & Edwards 1994; Walker & Schlacher 2014; Ng et al. 2017). These structures can act as substrates which facilitate coral recruitment or function as solid platforms for the transplantation of coral fragments (Chavanich et al. 2014; Boström-Einarsson et al. 2020a). Once covered by corals, they can enhance coral recruitment to surrounding areas via sexual or asexual reproduction (i.e., the release of larvae by, or fragmentation of, the coral colonies located on the structures) or by "attraction" of settlement by cues associated with the corals (Edwards & Clark 1999; Gleason et al. 2009; Shaish et al. 2010). Recently, a project combining the use of inexpensive modular structures and coral transplantation to remediate reefs affected by blast fishing in Indonesia demonstrated that coral rehabilitation can be achieved over large spatial scales (Williams et al. 2019). Alternatively, small artificial structures seeded with coral larvae can be relocated to degraded areas where causes of ill health have been treated (e.g., eutrophication reduced, herbivores protected) to enable rapid reseeding (Chamberland et al. 2017). This could produce a domino effect, accelerating natural recovery across a larger area.

When deployed as coral recruitment substratum, the success of artificial reefs highly depends on the efficiency of materials and surfaces to encourage the spontaneous settlement of coral larvae, as well as the subsequent survival and growth of coral settlers (Yanovski & Abelson 2019). Physical characteristics of artificial substrates can affect biophysical drivers influencing recruitment dynamics. These include the composition of the material, their surface texture, color, chemistry, stability and durability, their size and orientation, and the shelter and shading that they offer (Spieler et al. 2001; Doropoulos et al. 2016; Nozawa et al. 2011). However, there is still a lack of research concerning the optimal physical parameters of recruitment substrates, so in many cases only the 'best guess' is available in terms of reef restoration methods (Spieler et al. 2001; Randall et al. 2020).

Various artificial materials have been tested as coral recruitment substrates, including ceramic, PVC, concrete, metal, bricks, wood, car tires, gabbro, granite, fiberglass, and coal ash (Harriott & Fisk 1987; Fitzhardinge & Bailey-Brock 1989; Lam 2003; Loh et al. 2006; Creed & De Paula 2007; Field et al. 2007; Burt et al. 2009; Mallela et al. 2017). Some of these materials can trigger higher recruitment than on adjacent natural reefs (Chansang et al. 2008). However, the majority of artificial reefs and coastal structures are made from concrete (Boström-Einarsson et al. 2020a), and novel materials and technologies have been hardly explored. For example, 3D printing technology allows one to create and replicate any shape with many different materials, that can be perfectly tailored to fulfill a biological function and serve as potential artificial reef structures (Mohammed 2016; Pioch et al. 2020; Ly et al. 2021). Biomaterials have emerged as alternatives to oil-sourced materials to prevent the spread of microplastics into the food chain. Owing to their biodegradability and non-toxic potential, a number of these materials, such as chitosan and chitin, could be used for multifunctional applications in the marine environment (Joseph et al. 2020). Finally, multi-layered concrete with different types of microstructures can be produced using different particle sizes of crushed calcareous aggregate and casting techniques to improve their bio-receptivity (Morin et al. 2018). These novel technologies and materials could be used on artificial reefs to promote coral recruitment.

In this study, we compared the efficiency of eight innovative and two commonly used materials as recruitment substrates for corals. Tiles made of the different materials were stacked in a vertical position and interspaced by 15 mm to create a cryptic environment. They were deployed for six months during major coral spawning events on the forereef of Mo'orea, French Polynesia. Thus, recruits were less than 6 months old. Material performance was compared using the cryptic sides of the tiles, where corals typically recruit (Arnold et al. 2010; Price 2010). Innovative materials, which varied in their durability, toxicity, environmental footprint and structural micro-complexity, were selected and tested for their potential applications in coral restoration. The two commonly used materials served as controls. We hypothesized that different materials influence coral recruitment. We also hypothesized that the benthic communities that colonize the materials affect coral recruitment success. The presence of some species of crustose coralline algae (CCA) is known to facilitate coral recruitment (Morse et al. 1996; Harrington et al. 2004; Vermeij 2005). In contrast, other benthic groups, such as bryozoans, macroalgae and sponges, can inhibit coral recruitment (Brandt et al. 2019). Consequently, we assessed benthic community development on the different materials and tested the relationships between coral recruitment and major benthic groups.

# 2. Materials and methods

## 2.1 Study site

Our study was carried out on the forereef between 7 and 13 m depth at a site called E2B ('Entre deux baies', 17°28.795'S 149°51.135'W) between Opunohu and Cook's Bays on the island of Mo'orea, French Polynesia (Fig. 5.1A-B). Mo'orea is an island with a circumference of 61 km and a surrounding lagoon and barrier reef of less than 2 km width (Adjeroud et al. 2007). The forereef of Mo'orea has repeatedly recovered from disturbances and is highly resilient (Kayal et al. 2012; Adjeroud et al. 2018). It is relatively free of direct anthropogenic disturbances and nutrient loads are low (Schrimm et al. 2004; Gil et al. 2016). Recruitment rates on Mo'orea's forereefs are relatively low compared to other Pacific reefs and are characterized by a dominance of Pocilloporidae and a low contribution of Acroporidae recruits, similar to those of high-latitude or sub-tropical Indo-Pacific reefs (Adjeroud et al. 2018).

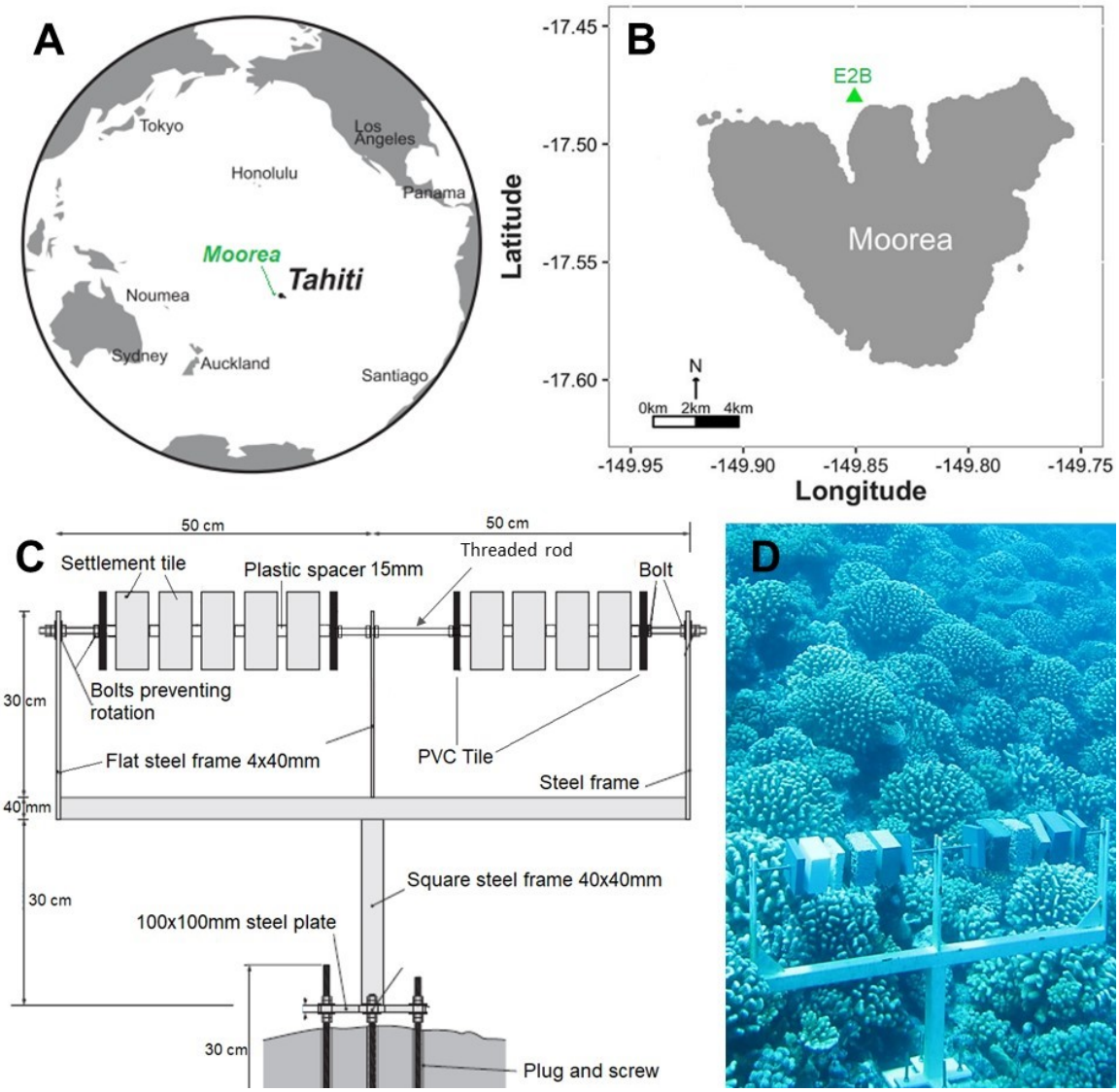
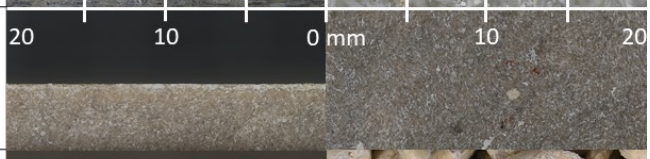
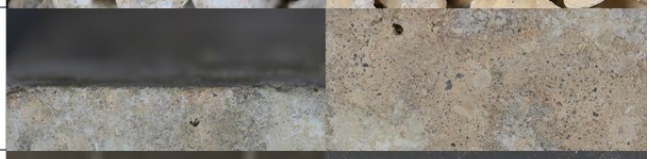
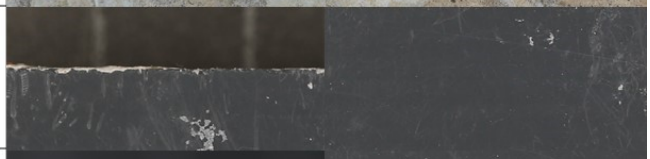
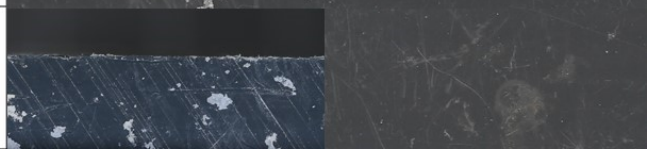



**Figure 5.1.** Location of study site in Pacific Ocean (A) and in Moorea (B). Layout of experimental unit (C) and photograph of its deployment on the forereef of Moorea (D).

## 2.2 Tested materials

Ten different materials were tested as recruitment substrates (see Table 5.1 for detailed descriptions and photographs of each material). Polyvinyl chloride (PVC) is widely used in coral settlement studies (Soong et al. 2003; Price 2010; Mallela et al. 2017). This material supports benthic communities similar to the natural substrate (Adey & Vassar 1975; Hixon & Brostoff 1996) and was considered as a control. Portland concrete is commonly used in marine construction and was considered as a second control. The other 8 materials were tested for the first time or have been rarely used in settlement studies. PVC CC consisted of PVC coated with a chitosan polymer. Chitosan is a linear polysaccharide obtained from the deacetylation of chitin through sodium hydroxide (NaOH) or chitinase. Since it is a particularly durable biomaterial, it could offer an effective coating for marine structures. However, while it is considered a biologically safe food additive, it also has antibacterial and antifungal properties (Xing et al. 2016), which may deter coral recruitment or aid in coral survival by deterring benthic competitors. Polylactic acid (PLA) is a bio-sourced and biodegradable polymer mixed with natural flax fibers. Flax-based PLA is used in 3D printing and in sailing yacht construction, and has been considered as a recyclable alternative to fiberglass, with a lower environmental footprint (Le Duigou et al. 2014). It may offer an alternative to oil-sourced materials for marine applications. FGP (fiberglass polymer) is made of fiberglass-based polymers, which are commonly used in marine constructions and have a high durability in seawater, although they are subject to chemical degradation over numerous years (Kootsookos & Mouritz 2004). This material can be used to build large structures (e.g., gridshells) and thus could have applications in large-scale coral restoration. Aquaroche® is made from natural clay (feldspar), shaped and baked into ceramic that is chemically neutral (Aquaroche® 2020). There are no colorants, binders or hardeners added. It is porous (porosity 28.3%) and light, and contains many crevices. It is used in the aquarium trade to host a micro-fauna helping in filtering water as an alternative to living stones and can be produced in any shape and size. Ceramic foam is a very light and porous material based on alumina ceramic. Unlike other industrial ceramics, it is a food-grade material which does not leak heavy metals. It offers a high number of calibrated pores, and its surface pH is closer to neutral compared with that of traditional concrete. 3D printed concrete and porous concrete offer complex microstructures in the form of grooves, ridges, deep holes and pores. However, their production involves the addition of adjuvants whose influence on benthic colonization is unknown. We selected two types of 3D printed concrete from two different manufacturers with two different lateral groove sizes. Porous concrete was formulated using conglomerates of 5 to 10 mm in diameter to create crevices ranging from 1 to 10 mm in depth.

**Table 5.1.** Description of the 10 materials used as settlement tiles

| | |
|---|---|
| **Large 3D printed rough concrete (3D concrete L) -** 100x100x50 mm<br>9 grooves per square side, 1-4 mm deep |  |
| **Small 3D printed rough concrete (3D concrete S)** – 100x100x30 mm<br>12 grooves per square side, 2 mm deep |  |
| **Aquaroche®** – 100x100x24 mm<br>Porous, friable and light ceramic, imitating 'living rock', few holes (1-10 mm) |  |
| **Ceramic foam** – 100x100x50 mm<br>Alumina based ceramic 'foam', extremely porous (0.5-2mm) and light |  |
| **Fiberglass polymer (FGP)** – 100x100x4 mm<br>Fiberglass-based polymer, smooth |  |
| **Polylactic acid (PLA)** – 100x100x4mm<br>Polymer based on natural flax fibers with polylactic acid, smooth |  |
| **Porous concrete** – 100x100x50 mm<br>Conglomerate of small concrete pieces, many deep crevices (1-10mm) |  |
| **Portland concrete** – 100x100x50 mm<br>Common concrete, contains few small holes (1mm), smooth |  |
| **Polyvinyl chloride (PVC)** – 100x100x20 mm<br>Classic PVC, smooth |  |
| **PVC with chitosane coating (PVC CC)** – 100x100x20 mm. PVC coated with a chitin-based polymer, smooth |  |

## 2.3 Field experiment

Settlement tiles were squared 100 x 100 mm tiles of thicknesses ranging from 4 to 50 mm depending on material type (Table 5.1). They were pierced through the center and mounted onto rods (Fig. 5.1C-D). Each unit consisted of 13 settlement tiles, including 4 PVC tiles and one tile of each of the other 9 material types, strung in two groups onto a 100 cm long threaded stainless-steel rod. Each rod was mounted horizontally on a steel frame, resulting in tiles being

oriented vertically. Within each group, tiles were separated from their neighbors by a 15 mm PVC spacer to mimic cryptic habitats, which are known to be favorable to coral recruitment (Arnold et al. 2010; Price 2010). The sequence of tiles on each unit was determined haphazardly, except for the PVC tiles that were positioned at the ends of each of the two groups of tiles. Thus, the outer side of the PVC tiles was exposed to external effects, such as herbivory and currents, while the inner side of the PVC tiles and both sides of all other tiles were kept cryptic. Each group of tiles was prevented from rotating with stainless steel bolts. Ten units were built, carrying a total of 130 settlement tiles. In total, each material was tested with 2,000 $cm^2$ of cryptic surface, except for the PVC of which 3,400 $cm^2$ of cryptic surface was available for settlement because we had to discard 6 PVC tiles which could not be dismantled.

Units were deployed using scuba diving at the study site on 28$^{th}$ August 2019 for a period of 6 months. Therefore, in the present study, all recruits were less than 6 months old. This deployment period coincides with peak recruitment periods of major coral families in Moorea, i.e., September to March for Acroporidae corals, and December to March for Pocilloporidae and Poritidae corals (Adjeroud et al. 2007). The timing and duration of the deployment allowed sufficient time for corals to recruit, and for a diverse encrusting community to become established on the tiles. Units were interspaced by ~4 meters and attached to the reef bottom with four screws and plastic plugs sealed with concrete. They were removed one by one between March 9$^{th}$ and March 19$^{th}$ 2020. Rods were detached from the steel frames, transported in a cooler filled with seawater to the CRIOBE research station, and kept under natural light in seawater flow-through tables until examination.

## 2.4 Coral recruit survey and benthic community characterization

Because of their small size, coral recruits were counted by screening all tile surfaces using a Leica EZ4 HD dissecting stereo microscope. During screening, individual plates remained submerged. They were placed in individual containers, supported on a plastic grid that had a stainless steel screw bolt sticking upwards out of the center. A NIGHTSEA SFA Light Head blacklight and glasses equipped with yellow filters were used to facilitate the finding of the recruits. Especially in the earliest stages, newly settled corals are very small (ca. 0.5 mm in diameter), pale in color, and sometimes hidden. Under UV light, some recruits fluoresce green and thus are easier to locate, even when tucked into a crack or partially obscured by other organisms (Schmidt-Roach et al. 2008).

To characterize the benthos, tiles were photographed on both sides in high definition using a Canon EOS 6D and a macro 100 mm lens, while submerged in seawater in their individual containers. A virtual grid of 49 points was overlaid on the HD pictures using the software PhotoQuad (Trygonis & Sini 2012). The substrate type/organism under each of the 49 points where gridlines intersected was identified using the following categories: live CCA, dead CCA, non-coralline encrusting red algae (such as *Peyssonnelia* spp.), turf algae (filamentous algae), filamentous cyanobacteria, macroalgae (foliose, corticated or articulated calcareous algae),

bryozoans, encrusting foraminiferans, sponges, tunicates, bivalve mollusks, wormtubes, corals, dead organic matter and bare substrate. Macroalgae were further subdivided as: *Lobophora* spp., *Dictyota* spp., and other macroalgae.

Tiles were then soaked in a 3:1 solution of freshwater and bleach for 24 h, gently rinsed in freshwater, and dried for 48 h. Recruit skeletons were located and identified at the family level under the stereo microscope. Only 3 families can be reliably distinguished in the first year after settlement (Babcock et al. 2003). The following identification criteria were: (1) Pocilloporidae: solid coenosteum, prominent septa and prominent columella; (2) Acroporidae: porous coenosteum, prominent septa, no columella; (3) Poritidae: septa with prominent teeth; (4) Other: different families, and; (5) Non-identifiable: when the skeleton was partially removed during the bleaching process, damaged, or not developed enough to confidently classify it into one of the first four categories (See Fig. 5.S1 for representative photos of identified recruits). Only recruits counted before bleaching of the tiles were identified. Other coral skeletons were most likely dead before initial tile screening and were not added to the initial counts. Photographs for benthic community characterization were taken within 3 hours following removal of the tiles from the study site, while screening of coral recruits before tile bleaching was conducted within 24 hours.

## 2.5 Statistical analysis

Recruitment analyses were conducted for all families combined and for the two most abundant families: Pocilloporidae and Acroporidae. Material performance was compared using the cryptic sides of the tiles, with each side considered a statistical unit. Recruitment data were over-dispersed and corresponded to neither a normal nor a Poisson distribution, so a negative binomial generalized linear mixed effect model (GLMM) with a log link was used to test for the effects of material type (explanatory variable) on coral recruitment (response variable) using the *Glmm.tmb* function of the *MASS* package (Venables & Ripley 2002) in R (R Core Team 2020). Unit was included as a random factor. The *Anova* function of the *car* package was run on the model to determine if the differences in recruitment were statistically significant at $p < 0.05$ (Bates et al. 2007). Tukey posthoc tests were conducted to detect differences in recruitment between pairs of materials using the *glht* function of the *multcomp* package (Hothorn et al. 2008).

To explore variations in benthic communities, we kept the 9 most abundant benthic categories (mean > 3% cover) and pooled the remaining into a $10^{th}$ category called 'other'. A PERMANOVA was used to determine differences in benthic community composition among material types using the *adonis2* function of the *vegan* package (Oksanen et al. 2009). Post-hoc PERMANOVA pair-wise tests were conducted to detect differences in benthic composition between material pairs. A principal component analysis (PCA) was performed to visualize variations in benthic community composition on the different materials using the *prcomp* and *autoplot* functions of the *ggfortify* package (Tang et al. 2016). The influence of individual benthic communities on

coral recruitment was examined by plotting recruitment against the percent cover of each benthic category. Both linear and logarithmic model curves were fitted using the *geom_smooth* function of the *ggplot2* package (Wickham 2016). Their significance was assessed using Pearson correlation tests with the *cor.test* function. Exponential model curves did not yield any significant results and thus are not shown. Negative binomial GLMMs were run incorporating all 10 benthic categories and unit as a random factor. The best model was selected with the stepAIC function of the MASS package (Venables & Ripley 2002). Negative binomial GLMMs incorporating the first and second principal components of the PCA produced higher Akaike information criterion (AIC) scores and thus are not shown. All data analyses were performed using R (R Core team 2020, version 3.6.1) in R Studio version 1.3.1073.

# 3. Results

## 3.1 Coral recruitment

A total of 848 coral recruits were recorded on the cryptic sides of the tiles, making an average of 3.96 recruits per 100 $cm^2$. 8.0% of these recruits were classified as non-identifiable. Of the recruits that could be identified, the large majority were Pocilloporidae (79.2%), followed by Acroporidae (11.8%), Poritidae (0.9%), and other families (0.1%). Coral recruitment varied significantly among tile materials (negative binomial GLMM Anova, $p < 0.001$; Table 5.S1). Recruitment ranged from 1.65 ± 0.55 recruits per 100 $cm^2$ (mean ± 95% CI) for Porous concrete to 5.65 ± 1.21 recruits per 100 $cm^2$ for Portland concrete (Fig. 5.2). Recruitment on Portland concrete, PVC and large 3D-printed concrete were significantly higher than on porous concrete and ceramic foam (Tukey's, $p < 0.05$). Aquaroche®, PLA, FGP, PVC, PVC with chitosan coating, and thin and large 3D concrete gave comparable recruitment (Tukey's, $p > 0.05$). Recruitment on Aquaroche® was significantly lower than on Portland concrete (Tukey's, $p < 0.05$). Recruitment of Pocilloporidae corals also differed significantly among tile materials (GLMM negative binomial Anova, $p < 0.001$; Table 5.S1), with a ranking of materials relatively similar to overall recruitment (Fig. 5.2). Acroporidae corals did not show a significant preference for any of the tested materials (negative binomial GLMM Anova, $p = 0.051$; Table 5.S1; Fig. 5.2).

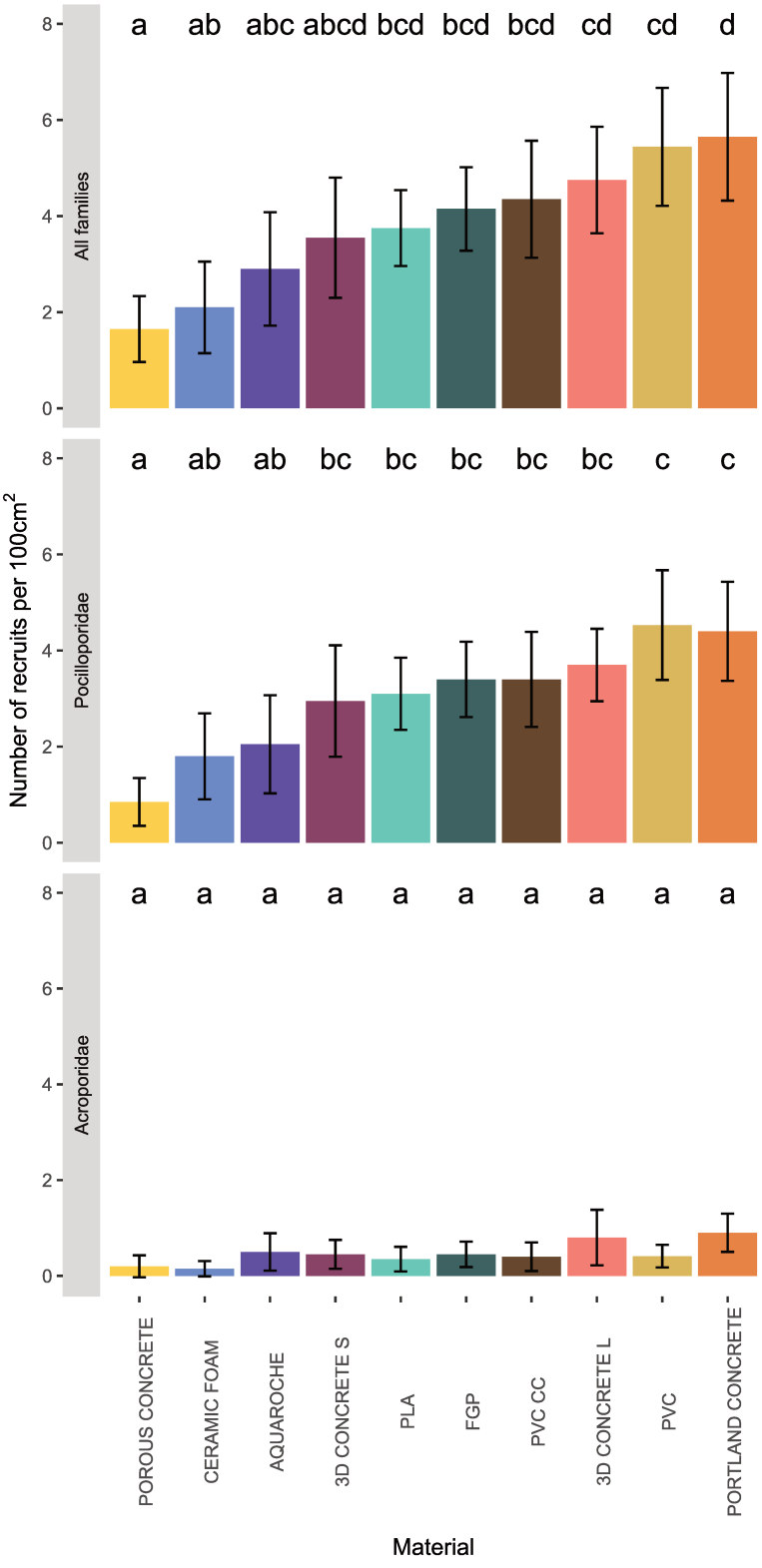



**Figure 5.2.** Coral recruitment (mean ± 95% CI) on the different materials for all coral families, Pocilloporidae, and Acroporidae. Letters indicate groups of materials with different recruitment based on Tukey's post hoc tests ($p < 0.05$). See Table 5.1 for material abbreviations.

## 3.2 Benthic communities

After six months of immersion *in situ*, settlement tiles were covered by diverse benthic communities (Fig. 5.3). Benthic community composition differed significantly among tile materials (PERMANOVA, pseudo-F = 7.9223, p = 0.001). Porous concrete had significantly different benthic communities than all other materials (Fig. 5.4; Table 5.S2). Encrusting red algae (21.5%) and sponges (11.4%) were particularly abundant on porous concrete. Aquaroche had a similar benthic community to ceramic foam and both 3D concretes. Bare substrate was particularly abundant on ceramic foam, as well as on Aquaroche® (14.4% and 13.0%, respectively). PLA had a similar benthic composition to PVC with chitosan coating, with the highest percentage of live CCA (33.6%), dead CCA (12.8%), and encrusting forams (7.1%). FGP had a similar benthic cover to PVC, and the highest *Lobophora* spp. cover (25.6%). Portland concrete had a similar benthic composition to that of both types of PVC.

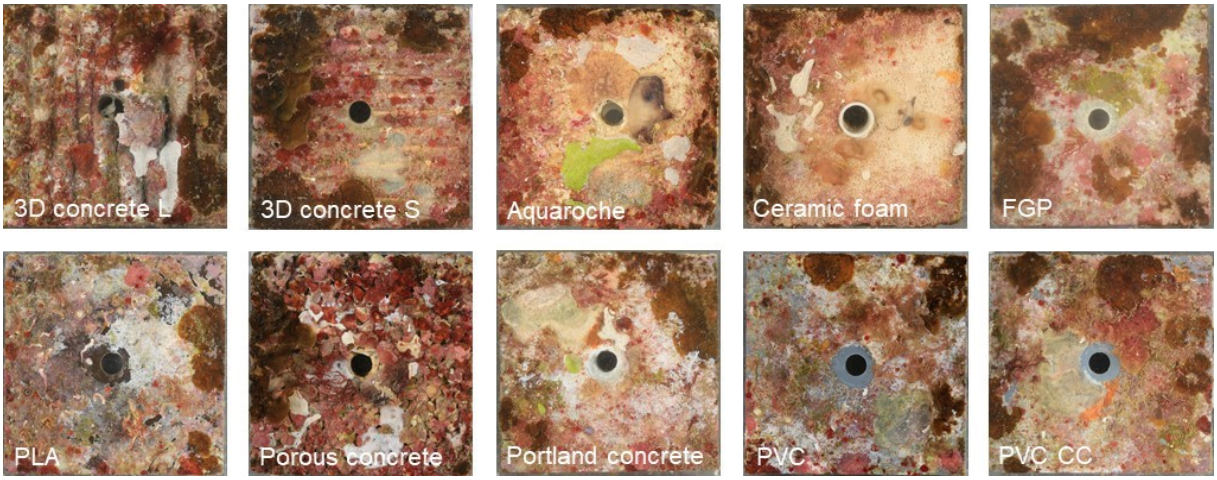



**Figure 5.3.** Representative tiles collected in the study, showing benthic communities on the different materials 6 months after immersion in the field. Benthic communities varied significantly among materials (PERMANOVA, pseudo-F = 8.44, p = 0.001). See Table 5.1 for material abbreviations.

## 3.3 Relationship between coral recruitment and benthic communities

Recruitment for all coral families was negatively correlated with the percent cover of bare substrate and encrusting red algae, with linear model curves showing a better fit in both correlations (Figs. 5.5A&C & 5.S2; Table 5.2). In contrast, recruitment was positively correlated with the cover of dead CCA, turf algae and encrusting forams (Fig. 5.5B, D & E). The model curve of recruitment as a function of dead CCA was significant with a logarithmic fit, suggesting that the positive influence of dead CCA on recruitment declined as dead CCA cover increased. Similarly, recruitment of Pocilloporidae corals was negatively influenced by the cover of bare substrate and encrusting red algae, and positively by dead CCA, turf algae and encrusting forams (Fig. 5.S3; Table 5.2). Bare substrate and bryozoans had a negative influence on recruitment of acroporid corals, with logarithmic and linear fits, respectively (Fig. 5.S4; Table

5.2). Although live CCA was a dominant benthic group (Fig. 5.4B), its cover did not correlate with recruitment for all families, Pocilloporidae or Acroporidae. When including all benthic categories in negative binomial GLMMs, the selected models indicated that recruitment for all families and Pocilloporidae were best positively predicted by forams and turf algae (Table 5.S3). Acroporidae recruitment was positively predicted by forams and *Lobophora* spp. (Table 5.S3).

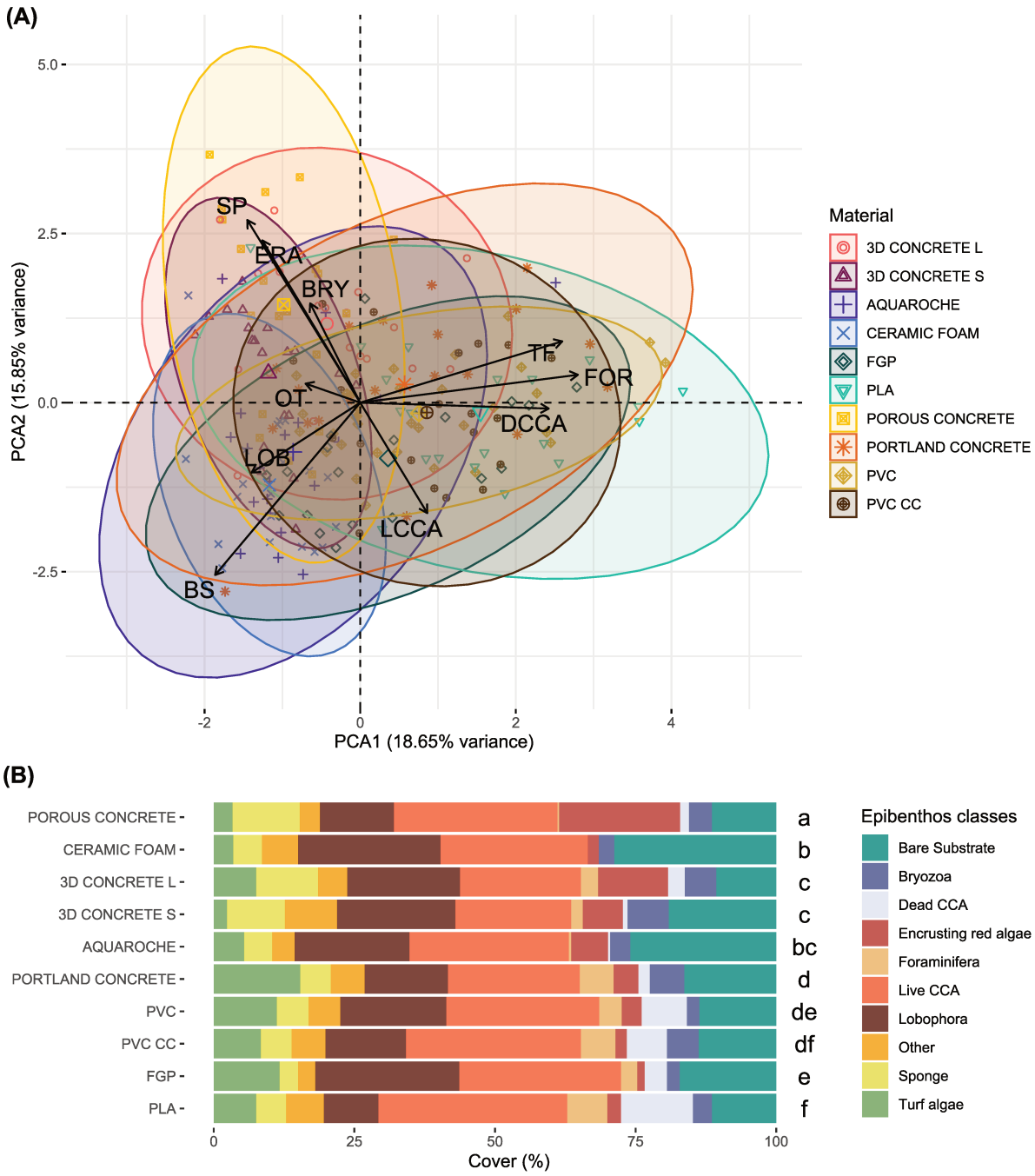



**Figure 5.4.** (A) Principal component analysis (PCA) plot showing the variation in benthic community composition among the different materials. (B) Percent cover of the dominant benthic categories on

the different materials. Letters (a-f) represent significantly different benthic compositions based on PERMANOVA pairwise posthoc tests (Table 5.S2). See Table 5.1 for material abbreviations.

**Table 5.2.** Results of Pearson's correlation tests between coral recruitment rates and the cover of the different benthic categories using linear and logarithmic fits. Significant correlations ($p < 0.05$) are in bold. When both fits are significant, the strongest correlation is highlighted in light grey.

| | | Linear model | | Logarithmic model | |
|---|---|---|---|---|---|
| | Benthic community | Correlation | p-value | Correlation | p-value |
| **All families** | Bryozoans | -0.056 | 0.413 | -0.0403 | 0.558 |
| | **Bare substrate** | **-0.207** | **$2.361*10^{-3}$** | **-0.189** | **0.006** |
| | **Dead CCA** | 0.095 | 0.168 | **0.194** | **0.004** |
| | **Encrusting red algae** | **-0.168** | **0.014** | **-0.136** | **0.047** |
| | **Foraminiferans** | **0.330** | **$8.073*10^{-7}$** | **0.412** | **$3.616*10^{-10}$** |
| | Live CCA | -0.060 | 0.385 | -0.036 | 0.603 |
| | *Lobophora* spp. | -0.042 | 0.538 | -0.016 | 0.811 |
| | Other | -0.105 | 0.124 | -0.100 | 0.144 |
| | Sponge | -0.028 | 0.688 | -0.043 | 0.534 |
| | **Turf algae** | **0.376** | **$1.333*10^{-8}$** | **0.361** | **$5.573*10^{-8}$** |
| **Pocilloporidae** | Bryozoans | -0.019 | 0.778 | -0.014 | 0.834 |
| | **Bare substrate** | **-0.147** | **0.032** | -0.127 | 0.064 |
| | **Dead CCA** | 0.093 | 0.175 | **0.178** | **0.009** |
| | **Encrusting red algae** | **-0.225** | **$9.283*10^{-4}$** | **-0.185** | **$6.570*10^{-3}$** |
| | **Foraminiferans** | **0.287** | **$2.023*10^{-5}$** | **0.388** | **$4.228*10^{-9}$** |
| | Live CCA | -0.060 | 0.385 | -0.043 | 0.530 |
| | *Lobophora* spp. | -0.060 | 0.380 | -0.035 | 0.608 |
| | Other | -0.080 | 0.246 | -0.086 | 0.208 |
| | Sponge | -0.062 | 0.365 | -0.065 | 0.342 |
| | **Turf algae** | **0.383** | **$6.781*10^{-9}$** | **0.372** | **$1.934*10^{-8}$** |
| **Acroporidae** | **Bryozoans** | **-0.141** | **0.039** | -0.106 | 0.122 |
| | **Bare substrate** | **-0.142** | **0.037** | **-0.155** | **0.023** |
| | Dead CCA | -0.015 | 0.828 | 0.021 | 0.754 |
| | Encrusting red algae | -0.002 | 0.974 | 0.022 | 0.747 |
| | **Foraminiferans** | **0.141** | **0.040** | **0.168** | **0.014** |
| | Live CCA | 0.032 | 0.640 | 0.058 | 0.396 |
| | *Lobophora* spp. | 0.032 | 0.645 | 0.032 | 0.640 |
| | Other | -0.075 | 0.273 | -0.041 | 0.554 |
| | Sponge | 0.091 | 0.184 | 0.045 | 0.512 |
| | Turf algae | 0.086 | 0.210 | 0.048 | 0.487 |

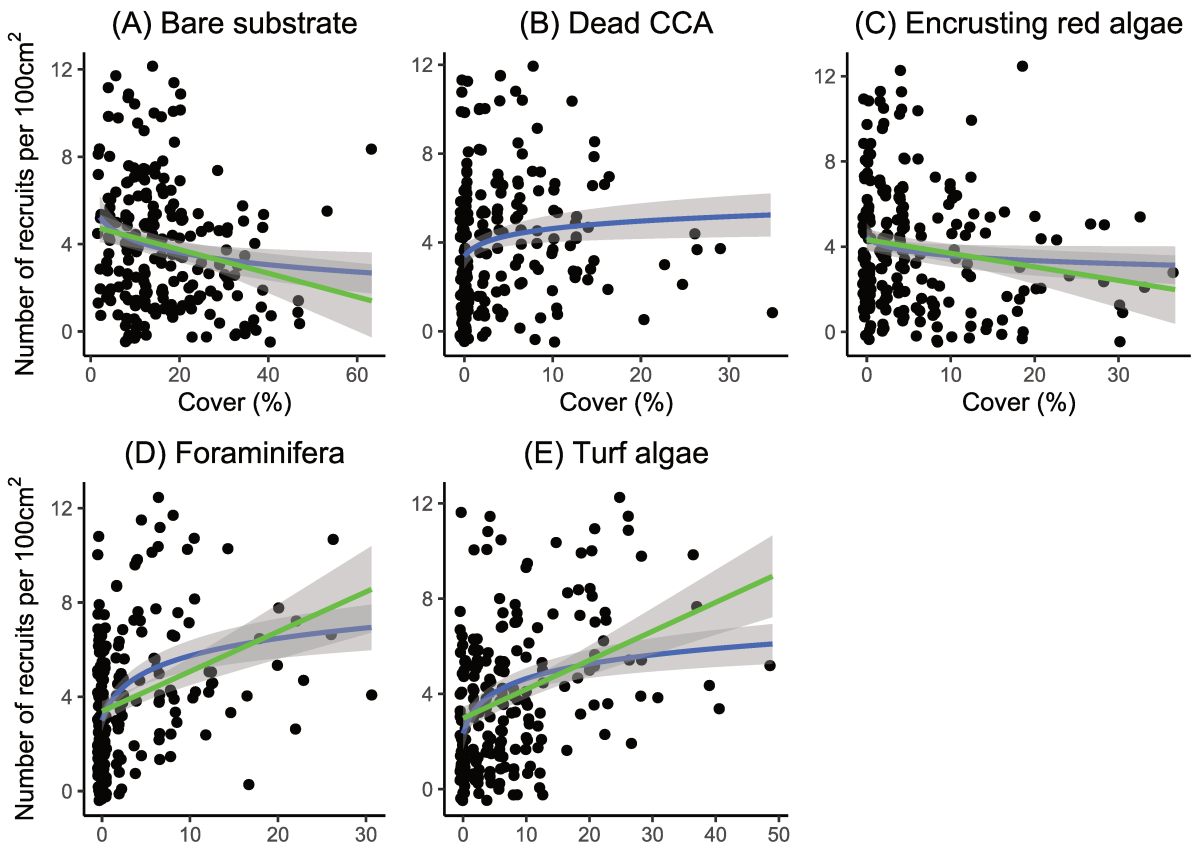



**Figure 5.5.** Relationships between coral recruitment rates (all coral families) and the cover of bare substrate (A), dead CCA (B), encrusting red algae (C), encrusting foraminifera (D) and turf algae (E). Dots represent raw data. All materials were pooled. Colored lines show linear (green) and exponential (blue) fits with 95% confidence intervals in grey shading. See Table 5.2 for significance values of each fit.

## 4. Discussion

This study set out to evaluate the efficacy of several innovative materials to act as recruitment substrates for corals. Material type significantly influenced rates of coral recruitment when combining all coral families, as well as early benthic community development. Six of eight innovative materials produced good recruitment (i.e., as high as those found on the control materials). After 6 months deployment on the forereef of Mo'orea, recruitment on control materials averaged 5.65 ± 1.21 and 5.44 ± 1.14 live recruits per 100 $cm^2$ when combining all coral families for Portland concrete and PVC, respectively, and 4.40 ± 0.93 and 4.53 ± 1.01 recruits per 100 $cm^2$, respectively, when only including the dominant family Pocilloporidae (i.e., ~3 months after spawning). These rates are high compared with similar studies conducted

at the same site. For example, recruitment ranged between one and four recruits per tile using horizontally oriented terracotta tiles (11 × 11 × 1 cm, all surfaces pooled) deployed annually between September and March over a period of 13 years (2001–2002 to 2013–2014) (Adjeroud et al. 2018). If we assume that all recruits were located on the cryptic side (i.e., underside) of the tiles, this makes a maximum of 3.3 recruits per 100 $cm^2$. Likewise, Price (2010) reported 1.0 ± 0.09 pocilloporid recruits per 100 $cm^2$ (mean ± SE) on cryptic surfaces of PVC tiles that were deployed between December 2003 and June 2004. The reasons behind the successful recruitment in our study are unknown, but it does not influence the reliability and validity of our performance test.

Coral recruitment on the two tested 3D printed concretes did not differ from those on control materials, suggesting that 3D printed concrete does not deter coral recruitment. This result is consistent with those of Ruhl and Dixson (2019) who reported that the coral *Porites astreoides* settled on 3D printed settlement tiles made of four different polymers (Ruhl & Dixson 2019). In their study, *P. astreoides* larvae settled at similar rates on the different 3D printed materials and the growth and mortality of recruits did not significantly differ between materials. Furthermore, the same study reported no alteration in the behavior of a coral-associated damselfish between 3D printed materials and natural coral skeletons. More recently, Randall and colleagues showed that corals successfully settled on PLA plastic 3D-printed grids (Randall et al. 2021). Together these results highlight 3D printing as a promising technology for coral restoration and our study expands on the number of materials that can be manufactured using this technology and successfully used as coral recruitment substrate.

Interestingly, we observed that recruits were often located at the groove bottom of 3D printed concretes. Corals often preferentially settle in micro-grooves of exposed surfaces (Petersen et al. 2005), and substrate with micro-ridges are more efficient in terms of growth of calcifying organisms (Mallela 2018). The choice to settle inside microstructures has been linked to higher survival rates of coral recruits due to e.g., lower competition with macroalgae and/or reduced accidental grazing by fishes (Nozawa 2008; Brandl & Bellwood 2016). However, our tiles were separated by a 15 mm gap which prevented access to fish and reduced the abundance of macroalgae. Therefore, we did not expect that recruits would retain this behavior in a cryptic habitat since it is less likely to increase their survivorship. Many factors other than grazing and macroalgae could influence coral recruitment within micro-crevices, including light and flow dynamics, boundary layers, and microbial communities (Babcock & Mundy 1996; Webster et al. 2004; Hata et al. 2017). For example, complex micro-structure is required to deliver coral larvae to the substratum even in calm flow conditions (Hata et al. 2017). Further research is needed on the importance and role of structural micro-complexity for coral recruitment in cryptic environments to elucidate the mechanisms driving this observation.

There was no significant difference in recruitment between PVC with and without chitosan coating, showing that chitosan does not deter coral recruitment and thus could be used as an effective coating for marine structures. Flax-based PLA and FGP also yielded recruitment that was not different than those of the controls. The suitability of flax-based PLA is in agreement

with the studies of Ruhl & Dixson (2019) and Randall et al. (2021) who reported successful coral recruitment on 3D printed tiles made of polymers. With less than 3 recruits per 100 $cm^2$, recruitment on Aquaroche® was significantly lower than one of the controls (Portland concrete) (Fig. 5.2). This material was brittle upon handling and showed signs of surface erosion at the end of the 6-month deployment period (Fig. 5.S5A). It displayed a high cover of uncolonized surface (i.e., bare substrate; Fig. 5.4A, brown plus signs), which could be caused by erosion. Surface erosion will simultaneously remove coral recruits and other benthic organisms. Likewise, Burt and colleagues showed that sandstone, which has a friable and light texture similar to Aquaroche® (although different in chemical composition), had the lowest number of coral recruits compared to ceramic, concrete, granite and gabbro tiles (Burt et al. 2009). Consequently, we do not recommend the use of Aquaroche® as recruitment substrate for coral restoration.

Two materials, ceramic foam and porous concrete, yielded significantly lower recruitment than the controls, suggesting that these materials are not suited as recruitment substrates for corals. Like Aquaroche®, ceramic foam proved to be fragile and prone to erosion (Fig. 5.S5B) and displayed a high abundance of uncolonized surface (Fig. 5.4A, green crosses). Furthermore, the small pore size of this material may not be ideal for the attachment of coral larvae and subsequent growth of recruits, and/or for the recruitment and growth of benthic organisms facilitating coral recruitment. Unlike ceramic foam, porous concrete had pores and crevices that were sufficiently large to allow recruits to grow. However, this material attracted non-coralline encrusting red algae, as well as sponges and bryozoans (Fig. 5.4A, blue squares). Previous studies have found the abundance of these benthic organisms to be negatively associated with coral recruitment on terracotta tiles (Arnold & Steneck 2011; Elmer et al. 2018). Thus, the rough and textured surface of porous concrete could have facilitated the settlement and growth of non-coralline encrusting red algae and invertebrate crusts which, in turn, inhibited coral recruitment. In our study, this is supported by the negative relationships between the cover of non-coralline encrusting red algae and coral recruitment (Fig. 5.5C), and between the cover of bryozoans and Acroporidae recruitment (Table 5.2).

The timing of deployment versus spawning pulses could have impacted taxa-specific recruitment patterns and their relationships with benthic communities. The *Acropora* species that spawned in September and October were exposed to early successional benthic communities and were subject to up to ~5 months post-settlement mortality when tiles were retrieved. In contrast, coral species that spawned later, such as from December to March for Pocilloporidae and Poritidae species, were exposed to more than 3 months conditioned tiles and had to survive less than 3 months before being retrieved. Natural succession to CCA dominance takes time and often goes through fleshy macroalgae and some turf and other taxa prior to reaching the more mature CCA stage (Adey & Vassar 1975; Arnold & Steneck 2011; Fabricius et al. 2015) Therefore, differences in the age of the benthic community at the time of spawning could have impacted taxa-specific settlement and post-settlement survival. Pocilloporidae recruits were generally larger than Acroporidae recruits on the tiles (Fig. 5.S6).

This suggests low settlement and/or high post-settlement mortality of early spawning *Acropora* species. A more frequent census of the tiles would have allowed us to study the relationships between the age of the benthic community and coral settlement and survival with more precision.

CCA cover is usually positively correlated with coral recruit density (O'Leary et al. 2012; Price 2010) and can enhance settlement rates (Morse et al. 1996; Harrington et al. 2004; Vermeij 2005). However, we did not find any significant relationship between live CCA cover and coral recruitment. Elmer and co-workers compared the settlement location of newly settled single coral polyps with benthic communities present on terracotta tiles and found that pocilloporid and poritid larvae changed their settlement preference depending on the age of the benthic community they encountered (Elmer et al. 2018). In their study, coral larvae reacted neutrally to CCA on terracotta tiles deployed for 3 months when bare space was still abundant but showed a positive settlement preference for CCA on tiles deployed for 9–15 months once bare space was limited. In our study, since tiles were retrieved after 6 months, this period may have been too short for the availability of CCA to positively influence recruit densities.

We found a negative relationship between recruitment and the cover of bare substrate. This is in contrast to other studies in which bare surface was a positive proxy for coral recruitment (Gouezo et al. 2020; Elmer et al. 2018). For example, pocilloporid larvae showed a preference to settle on biofilm on terracotta tiles deployed for 3 months or reacted neutrally to it on tiles deployed for 9-15 months (Elmer et al. 2018). In our study, the negative relationship between recruitment and the cover of bare substrate could have been driven by the mechanisms by which the different tested materials inhibited coral recruitment. As we explained above, two materials (ceramic foam and Aquaroche®) simultaneously had high cover of bare substrate (> 25% cover, Fig. 5.4B) and low recruitment (< 3 recruits per 100 $cm^2$, Fig. 5.2), which was most likely a result of surface erosion. Interestingly, there was a logarithmic relationship between coral recruitment and the cover of dead CCA. Prior research observed a clear preference of larvae to settle on live rather than dead CCA (Harrington et al. 2004; Siboni et al. 2020). Our study is the first to point out a positive correlation between dead CCA and coral recruitment in the field. This result suggests that a dead CCA patch acts as free space favorable to coral recruitment, but that its positive effect diminishes above a certain threshold (i.e., > 15% cover, Fig. 5.5B). Our tiles were colonized by one major type of encrusting foraminifera (see representative photographs in Fig. 5.S7). We found a strong positive correlation between coral recruitment and the percent cover of this organism (Fig. 5.5D). It is plausible that corals and encrusting foraminiferans rely on the same type of environment to grow and/or that encrusting foraminiferans facilitate coral recruitment. Foraminiferans contribute significantly to calcification on coral reefs, especially in cryptic environments (Mallela 2013).

Recruitment positively correlated with the cover of turf algae. Since this functional group has been shown to negatively affect corals at all life stages (Nugues & Roberts 2003; Birrell et al. 2005; Arnold et al. 2010), this relationship was unexpected. However, the effects of turf algae on corals are highly dependent on turf height and sediment load (Birrell et al. 2005; Mumby

et al. 2013; Ford et al. 2018). Long, dense algal turfs tend to trap sediment, impede water flow and create low-oxygen micro-environments (Carpenter & Williams 1993; Purcell 2000; Jorissen et al. 2016). Sediment accumulation in algal turfs has been shown to inhibit coral settlement (Birrell et al. 2005; Arnold et al. 2010). Jorissen and colleagues found that thin turf algae (less than 5 mm in height) did not overgrow corals as rapidly as thick turf algae (5–20 mm) (Jorissen et al. 2016). More recently, Randall and co-workers showed that coral spats survived well in the presence of turf inside open-wide grooves where sediment did not accumulate (Randall et al. 2021). In our study, tiles were vertically oriented which prevented sediment accumulation, and turf algae rarely exceeded 5 mm in height (CL & LH, personal communication). These algae were most abundant on three materials (Portland concrete, PVC, and FGP) that harbored similarly diverse benthic communities characterized by low abundances of recruitment inhibitors and yielded good recruitment.

The choice of materials for reef restoration necessarily involves some environmental and ethical considerations. In particular, practitioners are increasingly urged to use materials with a lower environmental impact for more sustainable restoration practices (Boström-Einarsson et al. 2020). For example, plastics accumulating on the oceans and beaches are a major threat to marine wildlife, including corals (Allen et al. 2017; Lamb et al. 2018). While PVC is commonly used in recruitment studies, it may not be easily accepted for large-scale restoration, regardless of whether it is coated with a biopolymer, such as chitosan. Likewise, natural fibres show a much lower $CO_2$ footprint relative to glass fibres (Le Duigou et al. 2014). Thus, flax-based PLA might be more acceptable than FGP for large-scale restoration. However, natural fibres suffer from low lifespan in the marine environment, which may limit their suitability for reef restoration. Similarly, the long lifespan of concrete in the marine environment reduces economic and environmental costs associated with frequent material renewal and makes it a long-term substitute for a rocky substrate. However, the production of concrete is a major source of greenhouse gas emissions (Worrell et al. 2001). Since its use for restoration directly contributes to climate change, the use of conventional concrete should be limited to specific engineering applications involving very small quantities of material. Interestingly, using a detailed cradle-to-gate life cycle assessment, Mohammad and colleagues showed that 3D concrete printing technology significantly reduced environmental impact of material construction as compared to conventional construction methods (Mohammad et al. 2020). Together with its success as recruitment substrates in our study, 3D printed concrete could thus allow the production of large structures with high complexity and low environmental footprint as compared to conventional concrete.

This study supports several innovative materials (3D printed concrete, PVC with chitosan coating, FGP and flax-based PLA) as promising materials for use on artificial reefs. It also suggests that the structural micro-complexity and durability of an artificial material and the composition of colonizing benthic communities can strongly influence coral recruitment. Since chemical (i.e., antifouling) and physical (i.e., porosity, robustness) effects will act simultaneously on sessile benthic communities, we cannot test for cause-and-effect

relationships that drove the low recruitment found on certain materials. However, this study highlights several physical (e.g., erosion) and biological (e.g., abundance and type of colonizing organisms) parameters that may influence coral recruitment on artificial materials. Due to the limited recruitment of Poritidae and other families, this study did not allow us to determine which artificial materials are most favorable to less common coral families like Poritidae, more threatened families like Acroporidae, or more generally highly diverse coral communities. More comparative approaches are needed in different geographic and environmental settings and under different experimental conditions (e.g., duration, exposure, structural micro-complexity) to better design well adapted artificial reefs. Importantly, while this study addressed the performance of innovative materials as recruitment substrates for corals, reactive restoration measures, such as the use of artificial reefs and structures, should not replace proactive management actions to protect natural habitats and to enable natural reef recovery (Boström-Einarsson et al. 2020a; Hein et al. 2020). Making natural substrates as suitable nursery substrates for corals using climate action and protection measures could limit the introduction of foreign materials into the marine environment and should remain a priority for the conservation and management of coral reefs.

## Acknowledgements

We thank the staff of the CRIOBE research station for logistical support. This study is supported by SEABOOST through the" Reef" Project and the Agence Nationale de la Recherche (No. ANR-18-CE02-0009-01). We acknowledge TotalEnergies R&D for funding this work and for giving permission to publish.

# 5. Supplementary information

**Table 5.S1.** ANOVA results of negative binomial generalized linear mixed effect models (GLMM) on recruitment rates for all families, Pocilloporidae and Acroporidae.

| | Fixed effect | Chi Sq | Df | Pr (>F) |
|---|---|---|---|---|
| All families | Material | 53.356 | 9 | < 0.001 |
| Pocilloporidae | Material | 55.507 | 9 | < 0.001 |
| Acroporidae | Material | 16.865 | 9 | 0.051 |

**Table 5.S2.** Pairwise PERMANOVA (adonis2) comparisons of epibenthic diversity covering different artificial substrata. P values are displayed above the diagonal, $R^2$ below the diagonal. Values displayed in red are significant (< 0.05).

| Material | 3D CONCRETE L | 3D CONCRETE S | AQUAROCHE | CERAMIC FOAM | FGP | PLA | POROUS CONCRETE | PORTLAND CONCRETE | PVC | PVC CC |
|---|---|---|---|---|---|---|---|---|---|---|
| **3D CONCRETE L** | | **0.278** | **0.194** | **0.001** | **0.003** | **0.002** | **0.04** | **0.046** | **0.023** | **0.022** |
| **3D CONCRETE S** | 0.068 | | **0.026** | **0.005** | **0.001** | **0.001** | **0.001** | **0.002** | **0.001** | **0.003** |
| **AQUAROCHE** | 0.081 | 0.119 | | **0.756** | **0.036** | **0.001** | **0.001** | **0.005** | **0.001** | **0.002** |
| **CERAMIC FOAM** | 0.235 | 0.142 | 0.030 | | **0.042** | **0.001** | **0.001** | **0.001** | **0.001** | **0.001** |
| **FGP** | 0.146 | 0.188 | 0.116 | 0.122 | | **0.002** | **0.001** | **0.021** | **0.066** | **0.036** |
| **PLA** | 0.236 | 0.318 | 0.294 | 0.392 | 0.217 | | **0.001** | **0.009** | **0.013** | **0.792** |
| **POROUS CONCRETE** | 0.127 | 0.251 | 0.285 | 0.418 | 0.334 | 0.327 | | **0.001** | **0.001** | **0.001** |
| **PORTLAND CONCRETE** | 0.114 | 0.174 | 0.194 | 0.271 | 0.137 | 0.146 | 0.271 | | **0.205** | **0.144** |
| **PVC** | 0.093 | 0.159 | 0.194 | 0.239 | 0.078 | 0.103 | 0.266 | 0.056 | | **0.421** |
| **PVC CC** | 0.137 | 0.193 | 0.222 | 0.265 | 0.120 | 0.027 | 0.288 | 0.086 | 0.037 | |

**Table 5.S3.** Results of best GLMMTMB (selected by stepAIC) of the influence of benthic categories on coral recruitment, with experimental unit as a random factor.

| | Explanatory variables | Estimate | P value |
|---|---|---|---|
| All families | **(Intercept)** | **0.8786** | **< 0.001** |
| | **Foraminifera** | **1.3531** | **< 0.001** |
| | **Turf algae** | **1.2203** | **< 0.001** |
| Pocilloporidae | (Intercept) | -1.4430 | 0.0752 |
| | **Bare substrate** | **1.2085** | **0.0209** |
| | Bryozoan | 0.6487 | 0.1185 |
| | **CCA** | **1.2961** | **0.0395** |
| | Dead CCA | 0.5965 | 0.1271 |
| | **Foraminifera** | **1.6955** | **< 0.001** |
| | Lobophora | 0.7559 | 0.0712 |
| | Sponge | 0.6803 | 0.1197 |
| | **Turf algae** | **2.0668** | **< 0.001** |
| Acroporidae | (Intercept) | -4.6812 | 0.0603 |
| | Bare substrate | -0.3195 | 0.8134 |
| | Bryozoan | -0.6466 | 0.5120 |
| | CCA | 2.3466 | 0.1466 |
| | Dead CCA | 0.3273 | 0.7241 |
| | **Foraminifera** | **2.2615** | **0.0209** |
| | **Lobophora** | **2.7268** | **0.0388** |
| | ERA | 1.2767 | 0.1998 |
| | Sponge | 1.6628 | 0.0978 |
| | Turf algae | 1.1988 | 0.2549 |
| | Other | 1.3679 | 0.1616 |

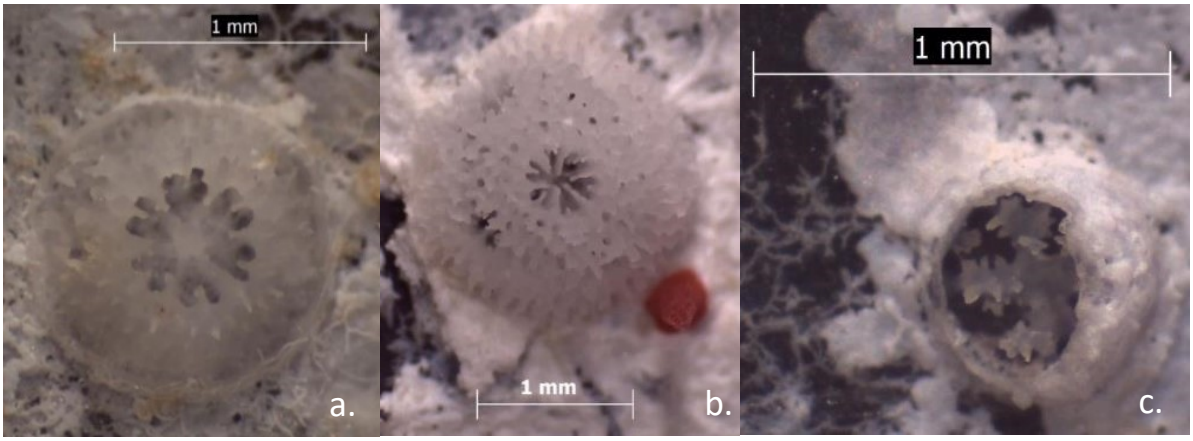



**Figure 5.S1.** Bleached skeletons of recruits identified as a. Pocilloporidae, b. Acroporidae, c. Poritidae

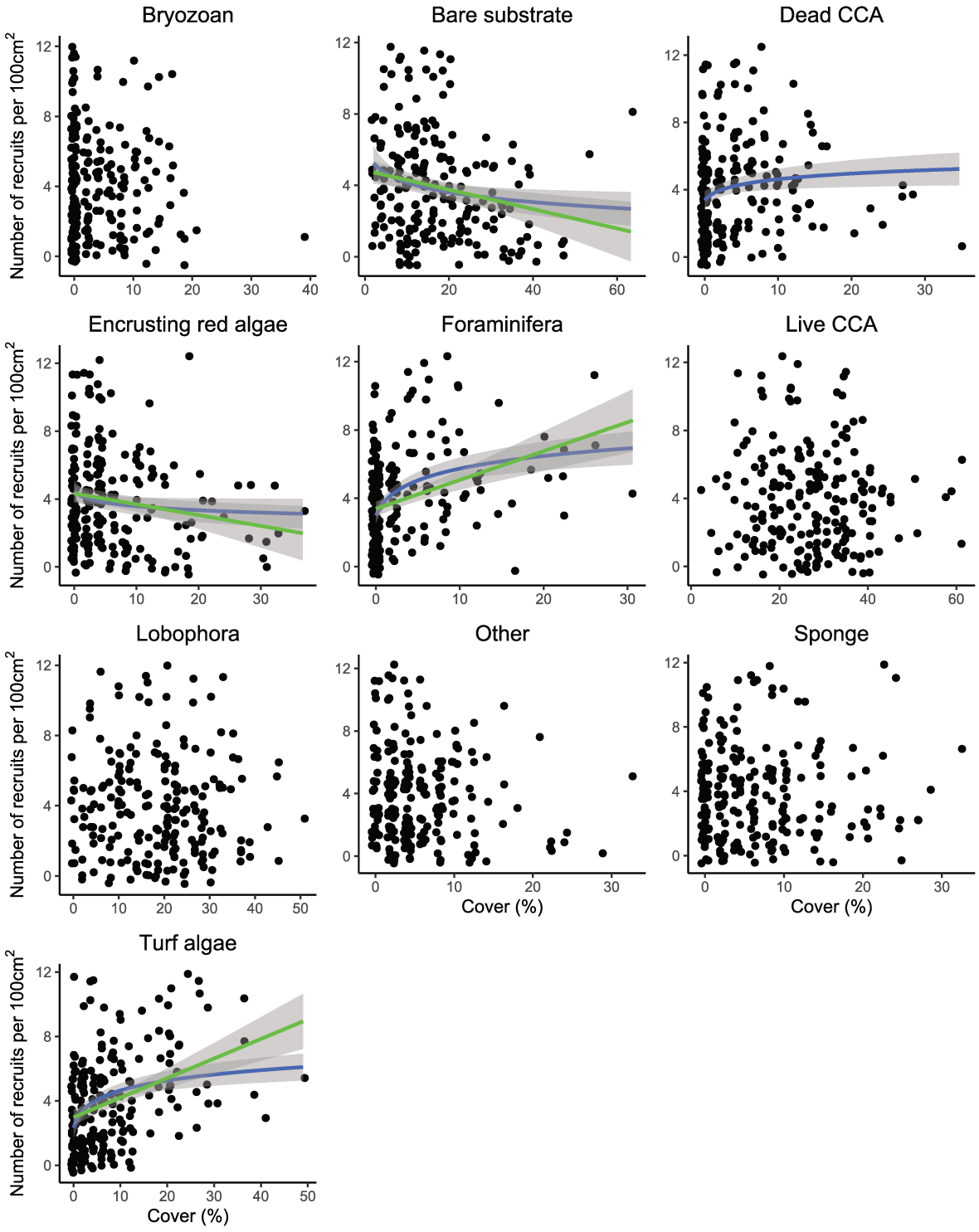



**Figure 5.S2.** Relationships between coral recruitment rate (all families) and the cover of the different benthic categories. Exponential (blue) and linear (green) fits are shown when there was a significant correlation, with grey shading representing 95% confidence interval. Only significant fits are shown (see Table 2).

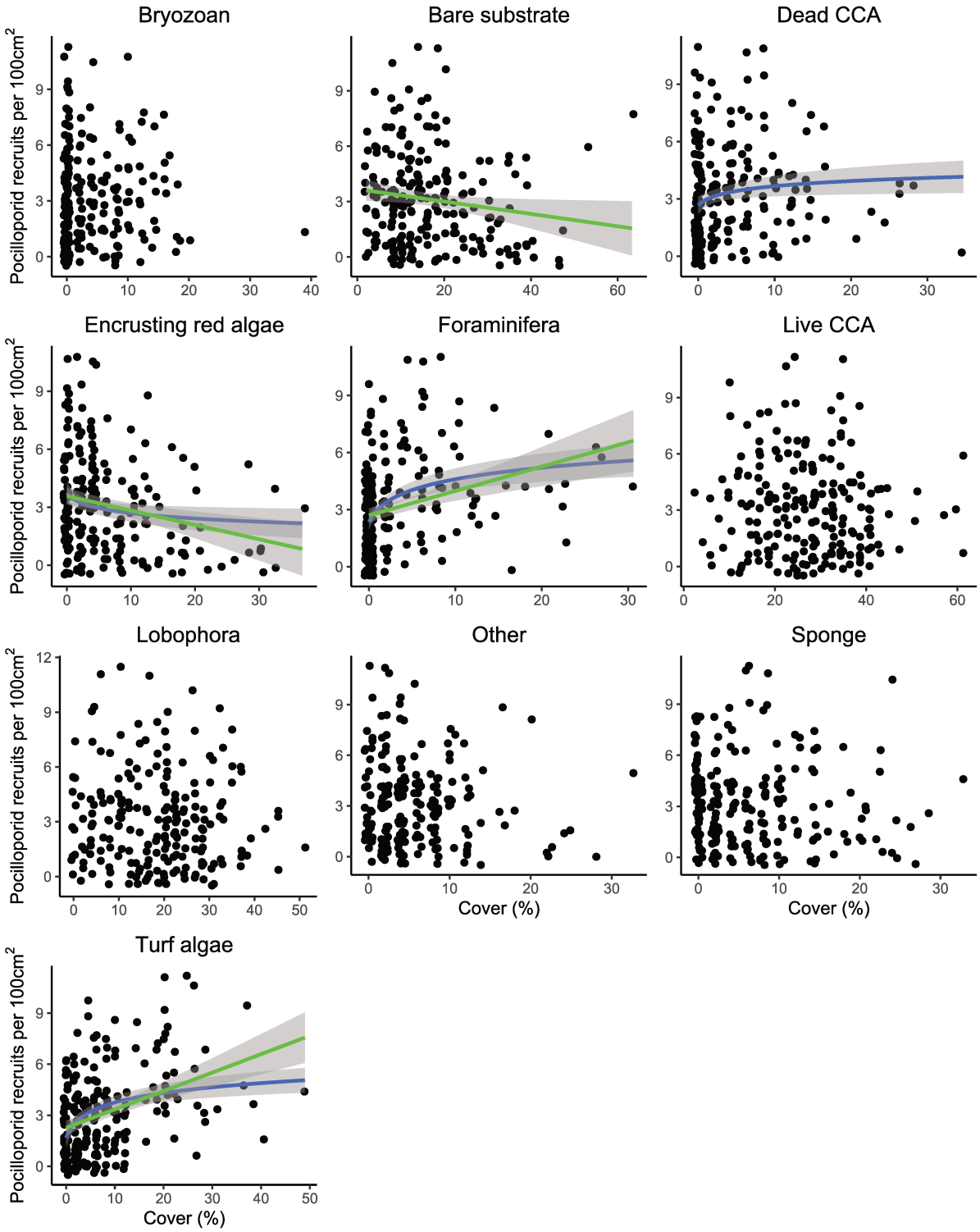



**Figure 5.S3.** Relationships between Pocilloporid recruitment rate and the cover of the different benthic categories. Exponential (blue) and linear (green) fits are shown when there was a significant correlation, with grey shading representing 95% confidence interval. Only significant fits are shown (see Table 2).

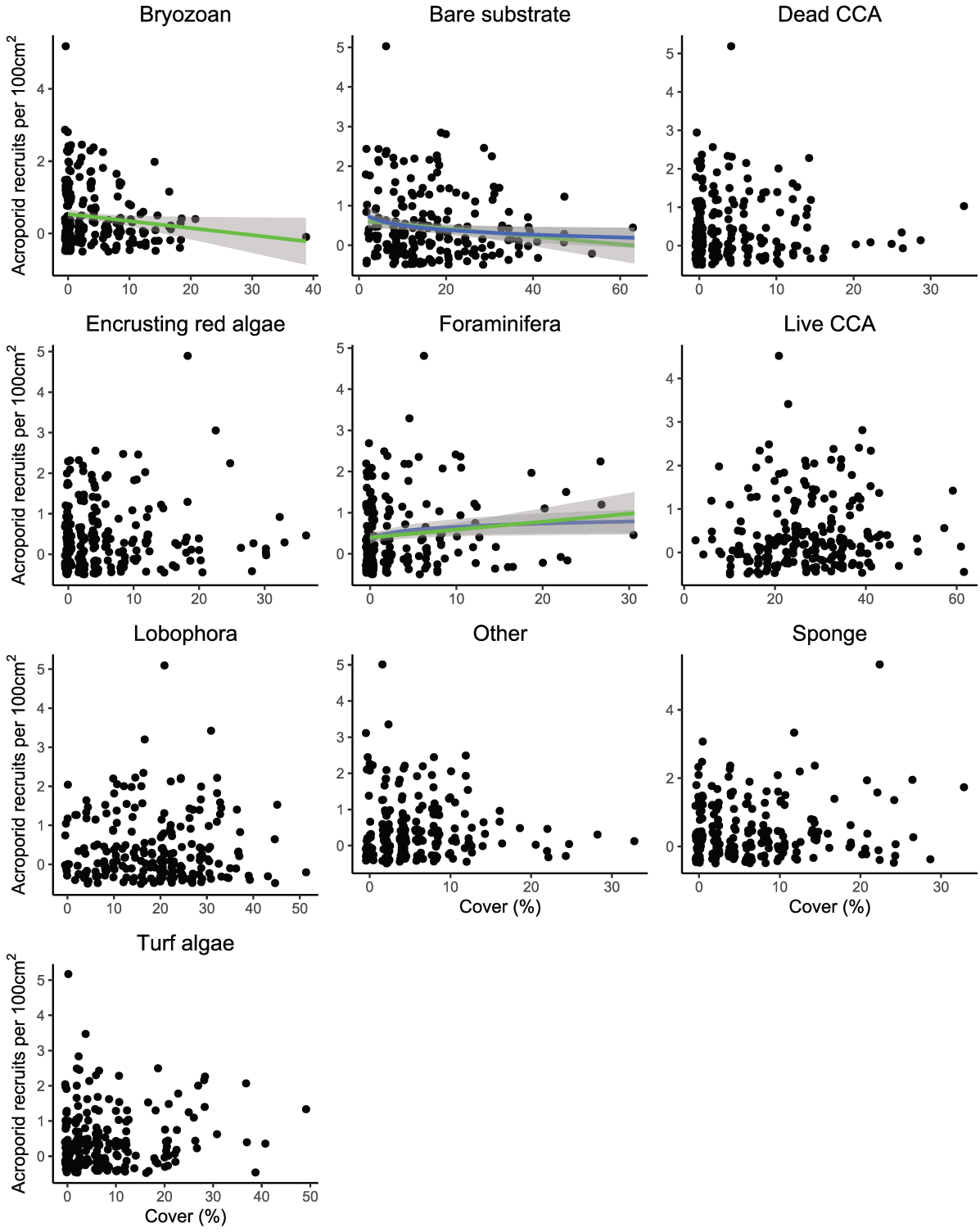



**Figure 5.S4.** Relationships between Acroporid recruitment rate and the cover of the different benthic categories. Exponential (blue) and linear (green) fits are shown when there was a significant correlation, with grey shading representing 95% confidence interval. Only significant fits are shown (see Table 2).

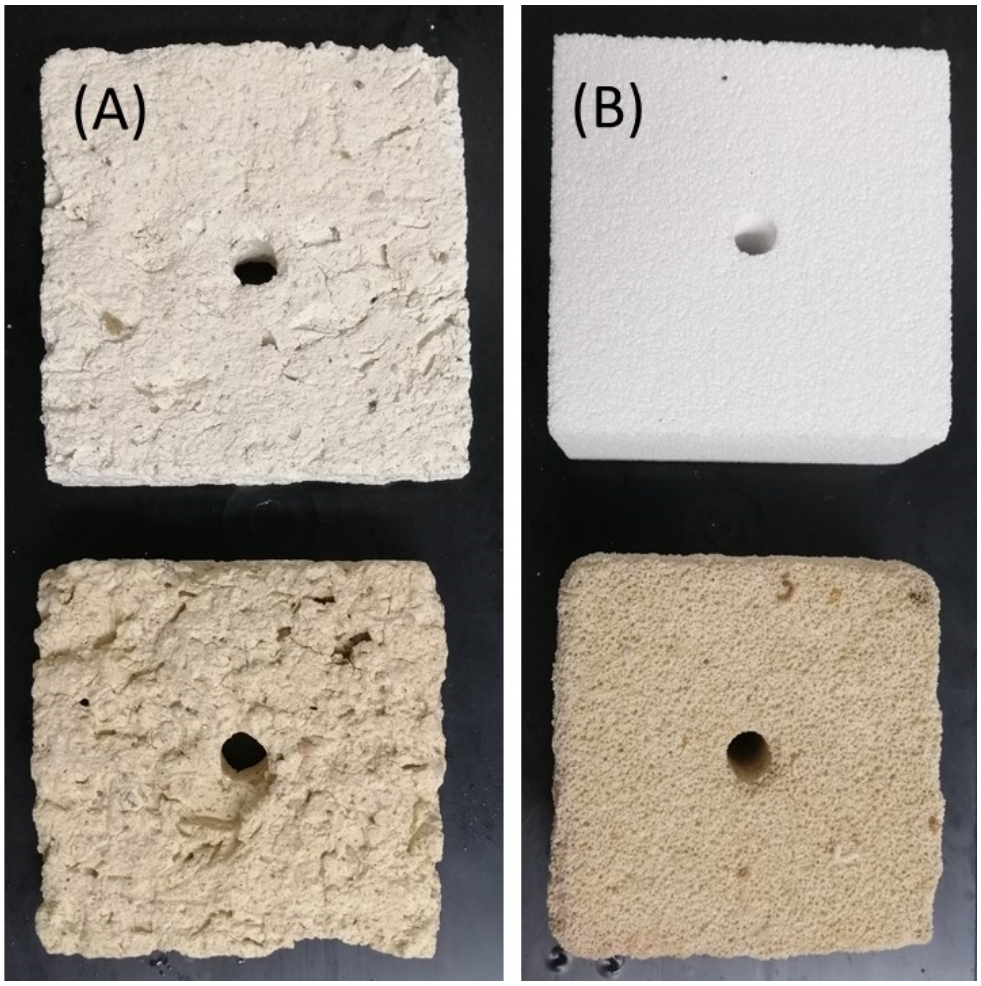



**Figure 5.S5.** Representative photographs of (A) Aquaroche® and (B) ceramic foam tiles before immersion (top) and after 6-month deployment in the field (bottom) following bleaching and removal of benthic organisms using water pressure. Tiles before immersion are 100 x 100 mm squares.

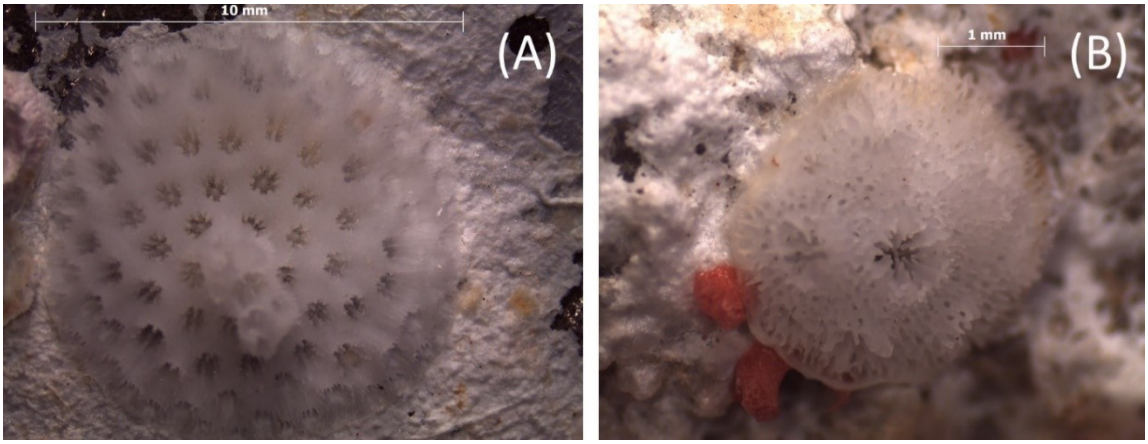



**Figure 5.S6.** Photographs of bleached skeletons of representative (A) Pocilloporidae and (B) Acroporidae recruits.

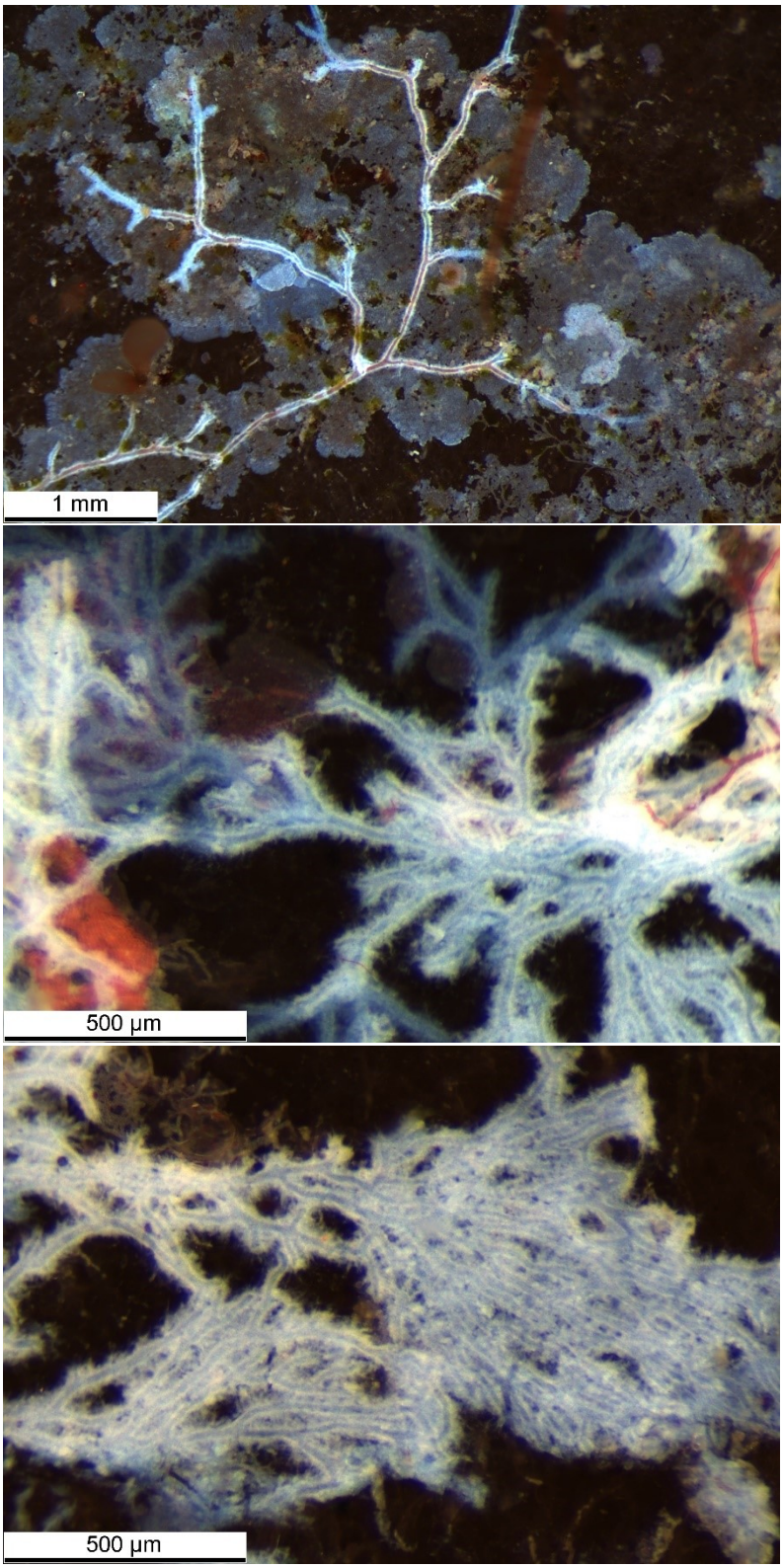
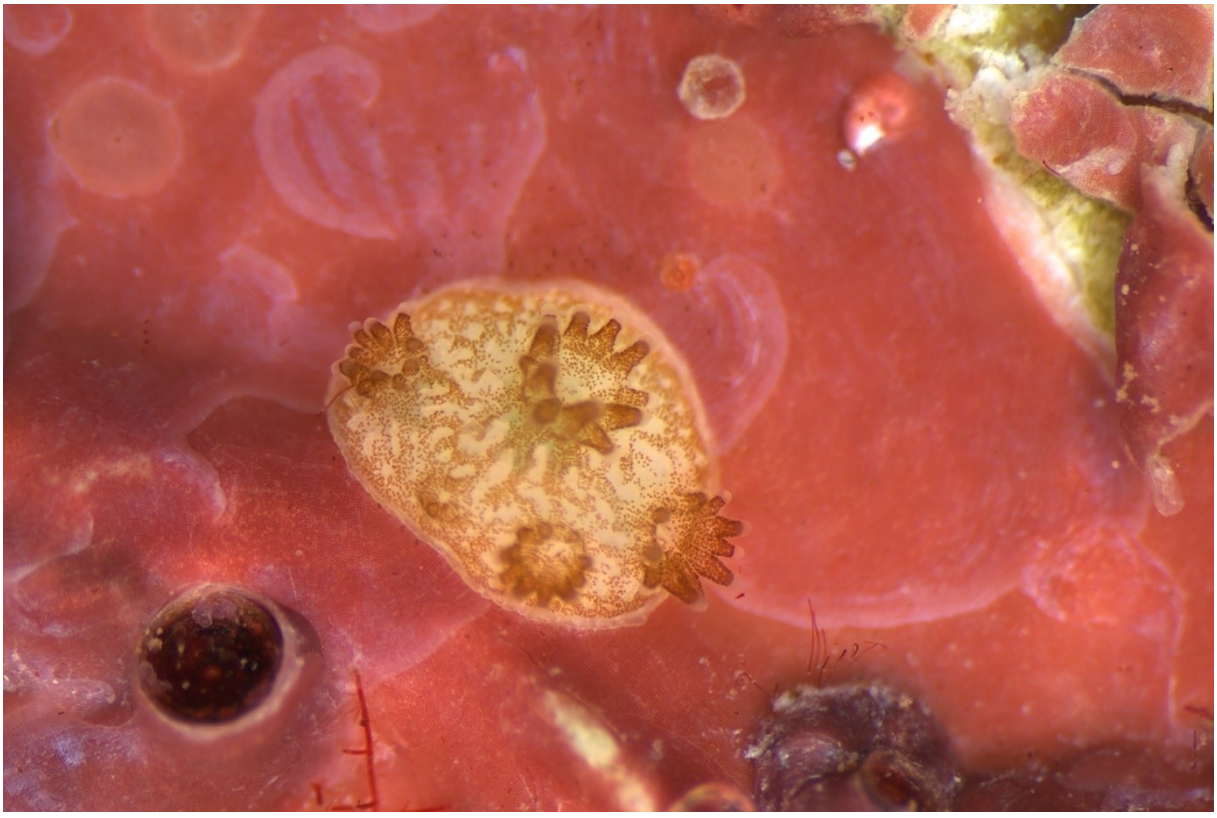



**Figure 5.S7.** Representative photographs of benthic organisms identified as encrusting colonial foraminifera.

# Chapter 6

## Optimizing surface complexity to enhance coral recruitment on artificial materials



# Optimizing surface complexity to enhance coral recruitment on artificial materials

**Running head:** Optimizing of surface complexities

*Camille Leonard[1], Laetitia Hédouin[1,2], Julie Stojakovic[1], Isabelle Davezies[1], Julien Dalle[3], Matthieu Lapinski[3], Philippe Blanc[4], Maggy M. Nugues[2,6]*

[1] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, BP 1013, 98729 Papetoai, Mo'orea, French Polynesia

[2] Laboratoire d'Excellence "CORAIL", Papetoai, Mo'orea, French Polynesia

[3] Seaboost, 889 rue de la Vieille Poste 34000 Montpellier, France

[4] TotalEnergies, Avenue Larribau, 64018 Pau Cedex, France

[5] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan, 52 Avenue Paul Alduy, 66860 Perpignan Cedex, France

## Abstract

Artificial materials are widely used as coral settlement substrates for aquaculture, coral restoration and scientific research. In order to optimize coral settlement and recruit survival, the chemical and physical characteristics of artificial materials and their influence on benthic colonization need to be better understood. The aim of our study was to investigate how porosity and the addition of crevices influenced natural coral recruitment on artificial materials. Settlement tiles composed of PVC or porous concrete, with or without added crevices of different shapes and sizes, were placed at 12 m depth in August 2019 on the fore reef of Mo'orea, French Polynesia. Settler density and size was assessed after nine months. The deployment was repeated in August 2020 but, after the 9-month assessment, tiles were replaced on the reef for one year to evaluate settler survival and growth. Adding crevices increased settler density after nine months on the tile upper sides, but decreased settler density on the undersides. Porous concrete showed high settler density after nine months regardless of the addition of crevices. Settler survival was low (6.1%), but it was highest on the upper sides of PVC tiles with added crevices. The benthos growing on the undersides and on porous concrete harbored high cover of *Lobophora*, which likely competed with coral settlers. A preference was found for squared crevices between 0.5 and 2 cm in width and depth. Our study emphasizes the importance of adding surface complexity on artificial materials to promote coral recruitment. Porous materials can lead to the colonization of unwanted benthic organisms and decrease recruit survival. In contrast, the addition of cryptic microhabitats on light-exposed surfaces optimizes recruit survival and growth.

# 1. Introduction

Reef-building corals are increasingly threatened by anthropogenic stressors, most importantly climate warming (Hughes et al. 2017b). While these invaluable organisms face high mortality because of rising sea temperatures, they are also confronted to local degradation by destructive fishing practices, man-made constructions and boat anchoring (Edinger et al. 1998; Fox & Caldwell 2006; Hughes et al. 2013; Flynn & Forrester 2019). Overall, global coral cover has declined by more than half since the 1950s, with an irreversible loss of over 63% of the associated biodiversity (Eddy et al. 2021). Hence, there is an urgent need for a more respectful coexistence of humans and coastal ecosystems. Restoration methods are increasingly being implemented as palliative solutions to improve coral reef resilience (Omori 2019; Boström-Einarsson et al. 2020a; Randall et al. 2020). Reef-building corals form three dimensional structures that increase the complexity of the ecosystem. In this regard, reef management objectives should aim to include the maintenance of structural complexity, as this promotes coral recruitment (Brambilla et al. 2021), provides refugia for numerous organisms and helps to maintain ecological processes (Graham & Nash 2013). In areas where corals have been physically destroyed, restoration projects aimed at either stabilising mobile substrate or creating new colonization surfaces can help the installation and recovery of biodiversity. To date, artificial reefs are now being implemented in virtually every coral reef region (Bracho-Villavicencio et al. 2023).

Artificial reefs have been shown to increase the habitat complexity of damaged areas and also enhance coral recruitment rates (Yanovski & Abelson 2019; Williams et al. 2019). These artificial structures can be specifically designed to fit the environment they are placed in and enhance local biodiversity. For instance, biomimetics can be used for a more efficient integration of structures in the ecosystem and for increased safety of artificial materials (Fabio et al. 2019; Giraldes et al. 2023). While artificial reefs are predominantly built from concrete (Vivier et al. 2021), different materials and textures have been trialed. Novel technologies such as 3D printing allow for various shapes and textures to be tested as potential artificial reef structures (Ly et al. 2021; Levy et al. 2022; Berman et al. 2023b). The physical and chemical characteristics of artificial substrates also need to be considered for efficient coral reef restoration projects. However, as fisheries enhancement is one of the most common objectives of artificial reefs (Vivier et al. 2021), very few are specifically designed to improve natural coral recruitment.

The settlement of coral larvae onto solid substrates can be influenced by biotic and abiotic factors, which include hydrostatic pressure, local currents, water soluble and insoluble chemicals, reef sound, temperature, photosynthetically active radiation, ultraviolet radiation and sedimentation (Gleason & Hofmann 2011). For instance, composition, surface texture, orientation, size and even color can influence artificial surface colonization (Spieler et al. 2001; Mason et al. 2011). Coral larvae have highly developed senses, being able to see light and colors (Babcock & Mundy 1996; Mason et al. 2011), sensing dissolved metabolites (Gleason et al. 2009) and reef sounds (Vermeij et al. 2010). Moreover, they are known to prefer rough

rather than smooth surfaces (Yanovski & Abelson 2019), cryptic rather than exposed surfaces (Mundy 2000), and certain biofilms and CCA species (Erwin et al. 2008; Jorissen et al. 2021). Since the biophysical drivers influencing settlement dynamics can vary across different coral families (Gouezo et al. 2020), the characteristics of artificial materials can be more beneficial for some families and less for others.

Often, less than 45% of settlers survive the first day post-settlement (Martinez & Abelson 2013). Post-settlement mortality rates are high because recruits are vulnerable to external pressures, such as predation by herbivores (Ritson-Williams et al. 2009; Christiansen et al. 2009), especially parrotfishes (Penin et al. 2010) or urchins (O'Leary et al. 2013), and competition with sessile organisms (e.g. sponges, ascidians, bryozoans) (Mundy 2000). It is hypothesized that the major factor influencing post-settlement community structure is predation (Spieler et al. 2001). For this reason, coral larvae often choose to settle into sheltered cracks, holes and other cryptic microhabitats (Mundy 2000). There is usually a positive correlation between structural complexity and benthic diversity (Spieler et al. 2001), and the availability of shelter is critical for successful recruitment. Coral recruit density and diversity are usually correlated with the irregularity of the substrate (Carleton & Sammarco 1987), which can be shaped by dead coral skeletons or parrotfish bite marks (McDevitt-Irwin et al. 2023). Consequently, the micro-complexity of an artificial material, such as added grooves and micro-crevices, can significantly enhance coral settlement rates (Nozawa et al. 2011; Edmunds et al. 2014), as well as recruit survival and growth (Nozawa 2008; Mallela 2018). Recruit survival is also enhanced on lower sides and on tiles with wider grooves (Randall et al. 2021). These refuge zones can significantly reduce grazing pressures on coral juveniles (Brandl & Bellwood 2016). Nonetheless, there is still a knowledge gap on how coral recruitment is affected by surface complexity.

The composition of benthic communities on artificial materials can affect the success of coral recruitment. For instance, coral larvae prefer to settle in grooves of artificial ceramic tiles, only when these are colonized by crustose coralline algae (CCA) and turf algae (Petersen et al. 2005). The presence of adult coral colonies and CCA are known to facilitate coral settlement (Morse et al. 1996; Heyward & Negri 1999; Vermeij 2005). Other benthic organisms such as *Aplysina* sp. sponges can in turn inhibit coral recruitment (Brandt et al. 2019). Macroalgae can also reduce coral settlement and increase the mortality rate of coral recruits through allelopathic effects (Fong et al. 2019; Harriott 1983; Bulleri et al. 2018; Beatty et al. 2018), hindering coral recruitment (McCook et al. 2001) and negatively impacting the microbiome of neighboring corals (Barott & Rohwer 2012). Moreover, coral recruits are easily smothered by turf algae that trap sediment particles (Birkeland et al. 1981). In this respect, the presence of grazing fish can be beneficial for coral recruitment by controlling algal densities (Brock 1979; Evensen et al. 2021). While many studies have examined the effect of different algae on coral settlement preferences, the effect of the benthos on coral recruitment and its context dependency with regards to factors, such as surface complexity, remain unclear.

This study aimed to test the effects of porosity and the addition of crevices on natural coral recruitment, settler size, survival, and growth. Settlement tiles were made of either a smooth (PVC) or a porous material (porous concrete), either flat or with crevices. Tiles were set up on the fore reef of the island of Mo'orea, French Polynesia in two field experiments. In the first experiment, coral recruitment was analyzed after nine months. The second experiment repeated the first experiment but, after the nine months' analysis, tiles were replaced on the reef for another 12 months to evaluate recruit survival and growth. Benthic community composition was characterized during each experiment. We hypothesized that different porosity and crevice characteristics would have varying influences on settler density, size, survival, and growth, and that some of these variations could be explained by varying colonizing benthic communities. For instance, we expected that porous materials with added crevices would harbor the highest settler density and best settler survival. Possible links between the physical traits of the substrates (i.e., porosity, crevice size/shape), the epibenthic communities and coral recruitment rates were also investigated. We expected high levels of CCA to have a positive influence, and high cover of macroalgae to have a negative influence on coral recruitment.

## 2. Material and methods

### 2.1 Field experiments

The present study was carried out on the fore reef of the island of Mo'orea, French Polynesia. The site called E2B ('Entre deux baies', 17°28.795'S 149°51.135'W) is located between Opunohu and Cook's Bays on the island (Fig. 6.1A-B). Two materials, polyvinyl chloride (PVC) and porous concrete (PC), were tested. Each have their own millimeter-scale substratum complexity owing to their different porosity. PVC is smooth, while PC is made of a conglomerate of small stones and cement and displays heterogenous pores mostly between 1 and 5 mm in width and depth (see details in Leonard et al. 2022, or chapter 5). Settlement tiles made from each material were squared 100 x 100 mm tiles with a thickness of 20 and 50 mm for flat PVC and other tiles, respectively. All tiles were pierced through the center to allow fixation with a bolt and nut.

Tiles with crevices were developed for each material to create a centimeter-scale complexity by adding crevices of four different sizes. Each upper and underside of the tiles with crevices had one crevice of 2 x 2 x 2 cm (length x width x depth), two crevices of 4 x 0.3 x 0.2 cm, six crevices of 1.2 x 1.2 x 1.2 cm and 13 crevices of 0.5 x 0.5 x 0.5 cm (Fig. 6.3C). As a result, each upper and underside had a total surface area of 98.54 and 165.46 $cm^2$ available for settlement for tiles without and with crevices, respectively, including crevice bottoms and vertical edges and after removing the central area covered by the bolt and nut. To deploy the tiles on the reef, 15 star-shaped units, each carrying four horizontal tiles (one of each material x crevice combination) 50 cm above the reef floor, were built (see Fig. 6.1C & D for the layout).

To initiate the first experiment, units were bolted on the reef between 11 and 14 m at E2B on 28th August 2019 and interspaced by 2-5 m. This deployment period coincides with peak recruitment periods of major coral families in Mo'orea, that is, September to March for Acroporidae corals, and December to March for Pocilloporidae and Poritidae corals (Adjeroud et al. 2007). Tiles were detached from the steel frames nine months later, between 26th May and 16th June 2020 in batches of three units (12 tiles) and mounted on ropes with 5 cm spacers to prevent contact between tiles. During boat transport, the ropes with tiles were placed in a cooler filled with seawater. Back in the laboratory, tiles were kept in seawater flow-through tables until analysis. In the first experiment, two tiles fell off a unit, thus the whole unit was omitted from analysis, leaving a total of 56 analyzed tiles.

For the second experiment, the same 60 tiles (4 x 15 units) were completely cleaned with a bleach bath, then an acidic bath (diluted HCl), then pressure washed with a Kärcher, and placed back onto the forereef on 28th August 2020. They were retrieved as described above and analyzed nine months later as in the first experiment (between May 18th and 28th 2021). However, unlike the first experiment, the tiles were redeployed at their exact location on the units and reanalyzed one year later (between May 10th and 27th 2022, i.e., 21 months since the start of the experiment). From retrieval to redeployment, tiles remained submerged and returned to the field within three days.

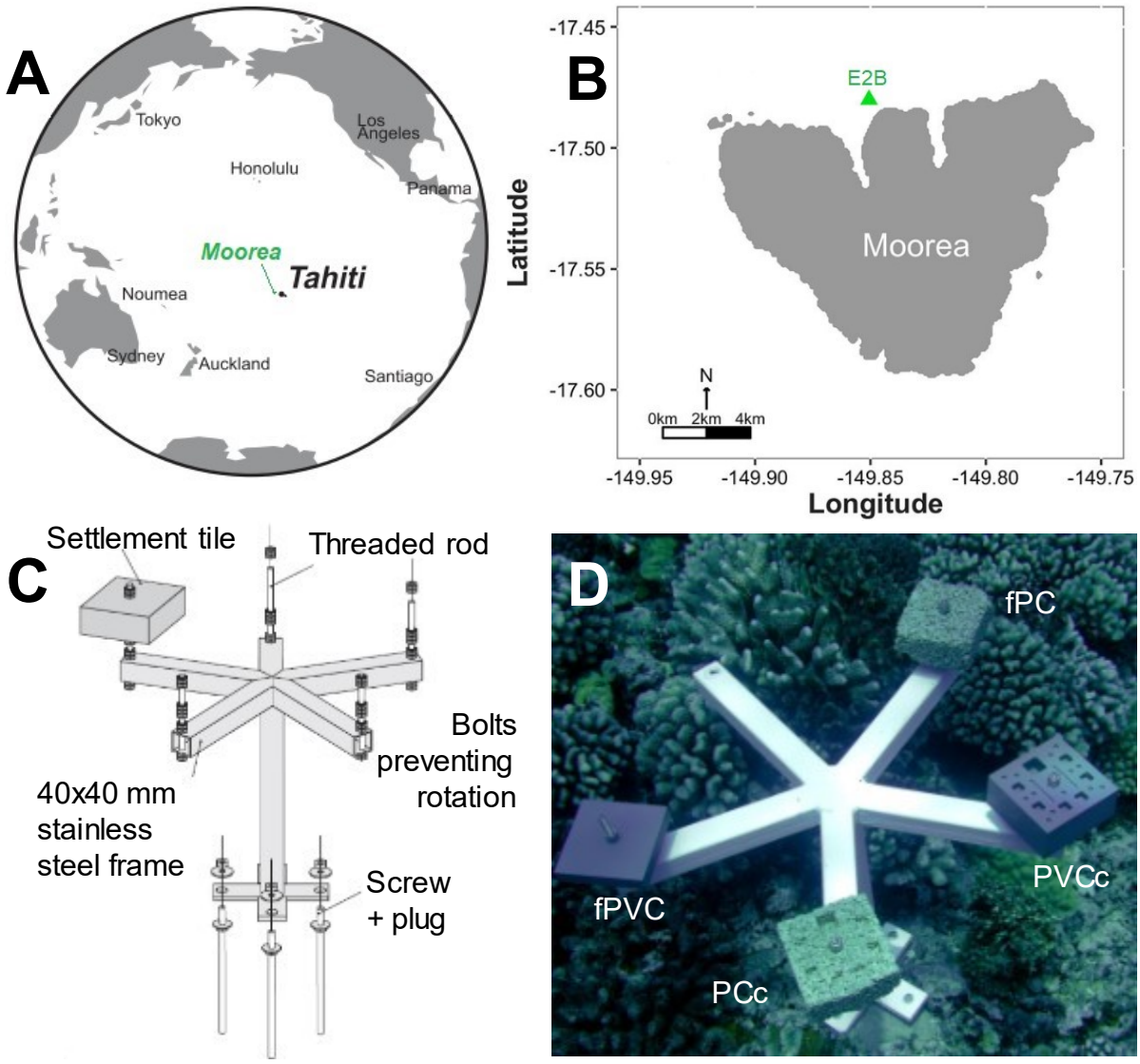



**Figure 6.1.** Location of study site in Pacific Ocean (A) and in Mo'orea (B). Layout of the experimental unit (C). Photo of experimental unit showing the four studied tiles (D); fPVC = flat Polyvinyl Chloride, PVCc = PVC with crevices, fPC = flat porous concrete, PCc = PC with crevices.

## 2.2 Coral recruit survey and benthic community characterization

In the laboratory, the upper and undersides of the tiles were photographed using a Canon EOS 6D equipped with a macro 100 mm lens in high definition, while being submerged in seawater in their individual containers. All coral settlers were counted by screening all tile surfaces using a Leica EZ4 HD dissecting stereo microscope. Tiles were placed in 15 x 15 x 6 cm containers filled with filtered seawater, resting onto a bolt with a vertical stainless-steel rod to create a gap and prevent the underside of the tile from being against the bottom of the container. A NIGHTSEA SFA Light Head blacklight and glasses equipped with yellow filters were used to facilitate the finding of the settlers. Under the blacklight, polyps usually fluoresce green and red and thus are easier to locate. All settlers were plotted onto the photograph using the

software PhotoQuad (Trygonis & Sini 2012). This allowed to know their exact positioning on the tile with respect to the different crevices and, for the second experiment, to determine the recruits that survived a year later and distinguish them from the new generation of settlers. Settler size was recorded as the number of live polyps.

To determine benthic community composition, a virtual grid of 49 points was overlaid on the photographs using PhotoQuad. The substrate type/organism under each of the 49 points was identified using the following categories: live CCA, dead CCA, encrusting red algae (ERA, such as *Peyssonnelia* sp.), turf algae (filamentous algae), cyanobacteria, macroalgae (foliose, corticated, or articulated calcareous algae), bryozoans, sponges, foraminiferans, solitary or colonial tunicates, vermetid snails, bivalves, serpulid worms, corals, dead organic matter and bare substrate. Macroalgae were further divided as: *Lobophora* spp., *Dictyota bartayresiana*, and other macroalgae. There are at least 37 species of *Lobophora* in French Polynesia (Vieira et al. 2023), thus we refer only to the genus.

After the analysis of the first experiment (2020), all tiles were bleached in a 3:1 solution of freshwater and bleach for 24 hours, then rinsed and dried for 48 hours. Settler skeletons were located and identified at the family level under the stereo microscope, as Acroporidae, Pocilloporidae, Poritidae, other families or non-identifiable (similar to chapter 5). During the second experiment (2021), settlers were not identified as they were kept alive to follow their growth and survival.

## 2.3 Statistical analysis

Settler density was expressed as the number of live recruits per 100 $cm^2$ of settlement tile surface, including crevice bottoms and vertical edges, but excluding millimeter-scale substratum complexity due to material porosity. Settlement data collected after nine months were pooled across experiments. The effects of material (2 level fixed effect, i.e., PVC and PC), crevices (2 level fixed effect, i.e., flat and with crevices), year (2 level fixed effect, i.e., 2020 and 2021 corresponding to the first and second experiments, respectively), side (2 level fixed effect, i.e., upper and under) and every possible interaction were tested using a generalized mixed effect model with a negative binomial distribution (*glmer*, *lme4* package) (Bates et al. 2015), and the *Anova* function from the *car* package (Bates et al. 2007). Unit was included as a random factor. Only interactions between year and other factors were omitted from the final model because they were not significant. The experiments were the same in both years, so the effects of side, material and crevices did not vary between years.

Settler survival was expressed as the number of all surviving settler in 2022 divided by all settlers in 2021 on each side and converted in %. Settler growth was expressed as the number of polyps at 21 months divided by the number of polyps at nine months (growth factor). Because there was no settlement on upper flat PVC tiles after nine months, the models for settler size, survival and growth were run separately for PC, tiles with crevices and undersides. On undersides, the material and crevices were set as fixed factors, on PC, the side and crevices

were fixed factors and on tiles with crevices, the side and material were fixed factors. Each time, the unit was set as a random factor. For settler size, the year was added as a fixed factor.

Since adding crevices enhanced coral settlement, we seek to determine which crevice type contributed to this enhancement by subdividing each upper and underside of the tiles into five areas (Fig. 6.3C). Settler densities on tiles with crevices were computed for each tile area and analyzed using a generalized linear mixed model with a negative binomial distribution with tile area, year, side and material as fixed factors and unit as a random factor.

To explore variations in benthic communities, we kept the seven most abundant benthic categories (mean > 1.5% cover) and pooled the remaining into two categories called “other fauna” and “other algae”. To test for the effects of side, material and crevices on benthic diversity after nine months (response variable), the count dataset was transformed using the *mvabund* function of the *mvabund* package (Wang et al. 2012). Then the function *manyglm* of the same package was used, with the benthic diversity as a response variable, the side, material and crevices as fixed factors, and a negative binomial distribution. Main effects and interactions were tested with the *anova* function. To test the fit of the models, the residuals vs. fitted plot was checked for random scattering.

Changes in benthic community composition (data from 2021 and 2022) were tested with *manyglm*, with the benthic diversity as a response variable (mvabund object) and the age (9 vs 21 months), side, crevices and material as explanatory variables. All possible interactions were tested. Temporal changes in the cover of individual benthic categories were also tested with a Kruskal-Wallis test.

The relationships of each benthic category and several recruitment variables (i.e., densities at nine months in 2020 and 2021, densities at 21 months, settler survival) were analyzed using Spearman’s correlation tests using the *cor.test* function for each orientation separately. All data analyses were performed using R (R Core team 2022, version 4.2.1) in R Studio version 2023.09.0. All scripts and raw data are available on GitHub (https://github.com/CamiLeonard/Surface_complexity).

# 3. Results

## 3.1 Settler density after nine months

Settler density after nine months was 2.8 times higher in 2021 than in 2020 (Fig. 6.2A & Table 6.S1A, $p < 0.001$). In 2020, 293 live settlers were counted on 56 settlement tiles, making an average of 1.98 settlers.100 $cm^{-2}$. In 2021, there were 867 live settlers on 60 tiles, making an average of 5.47 settlers.100 $cm^{-2}$. Settlers were composed of Pocilloporidae (68.3%), Acroporidae (24.5%), Poritidae (4.7%) and other families (2.5%). There were significant main effects of side and material on settler density, but both effects were dependent on the addition of crevices as indicated by the significant interactions side x crevices and material x crevices (Table 6.S1A). Settler densities on upper sides were increased by 43.2% on tiles with crevices (3.25 ± 0.51 settlers.100 $cm^{-2}$; mean ± SEM) relative to flat tiles (2.27 ± 0.77 settlers.100 $cm^{-2}$). In contrast, settler densities on undersides were reduced by 40.3% on tiles with crevices (3.71 ± 0.48 settlers.100 $cm^{-2}$) relative to flat tiles (6.21 ± 0.75 settlers.100 $cm^{-2}$). Settler densities on flat tiles were increased by 173.6% on undersides (6.21 ± 0.75 settlers.100 $cm^{-2}$) relative to upper sides (2.27 ± 0.77 settlers.100 $cm^{-2}$), while settler densities on tiles with crevices did not vary between side. With regards to material, settler densities on PVC were increased by 20.4% on tiles with crevices (3.48 ± 0.38 settlers.100 $cm^{-2}$) relative to flat tiles (2.89 ± 0.58 settlers.100 $cm^{-2}$), while no difference between with and without crevices occurred on PC. Settler densities on flat tiles were increased by 93.8% on PC (5.60 ± 0.94 settlers.100 $cm^{-2}$) relative to PVC (2.89 ± 0.58 settlers.100 $cm^{-2}$), while no difference between material occurred on tiles with crevices.

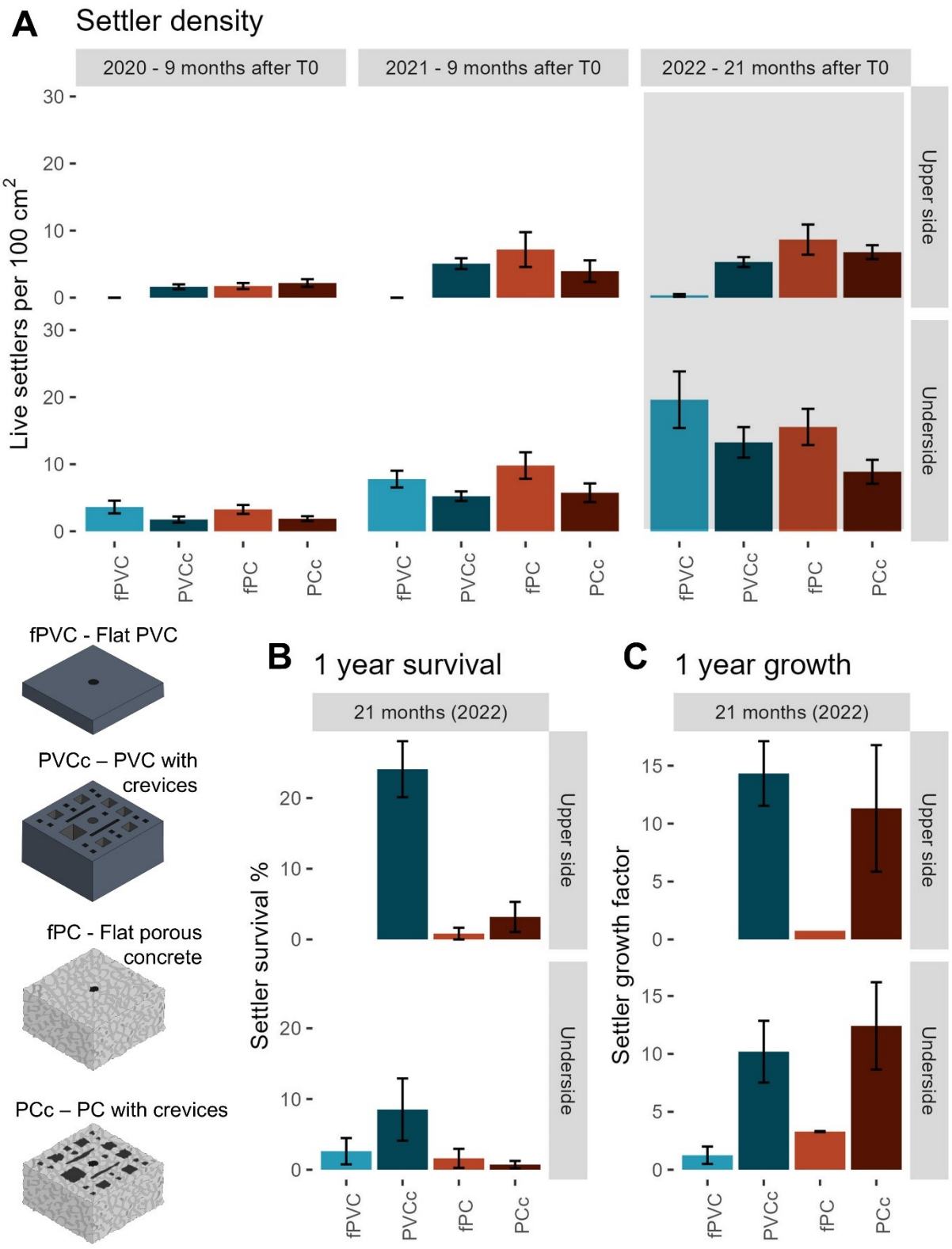



**Figure 6.2.** Mean (± SEM) density of coral settlers per 100 $cm^2$ in 2020 and 2021 after nine months in situ (A), and new settlement (between 2021- 2022), settler survival (%) (B), growth factor (final number of polyps/initial) (C) as a function of tile side, material and crevice addition: fPVC = flat Polyvinyl Chloride, PVCc = PVC with added crevices, fPC = flat porous concrete, PCc = PC with added crevices.

## 3.2 Settler size after nine months

Settler size after nine months on undersides was significantly influenced by material (Table 6.S2A, Fig. 6.S1). It was increased by 23.1% on PVC (2.98 ± 0.11 polyps) relative to PC (2.42 ± 0.08 polyps) on undersides. There was a significant effect of side and crevices on settler size on PC (Table 6.S2A). The effects of side and crevices were dependent on each other, as indicated by a significant interaction side x crevice on PC tiles (Table 6.S2A). Settler size on upper PC surfaces was increased by 57.3% on tiles with crevices (4.01 ± 0.31 polyps) relative to flat tiles (2.55 ± 0.14 polyps), while settler size on PC undersides did not differ with crevice addition. Settler size on PC tiles with crevices was increased by 63.8% on upper sides (4.01 ± 0.31 polyps) relative to undersides (2.45 ± 0.12 polyps), while settler size on flat PC tiles did not differ between tile side. There was a significant effect of side and material on settler size on tiles with crevices (Table 6.S2A). Settler size on tiles with crevices was 20.7% larger on upper sides (3.38 ± 0.13 polyps) than on undersides (2.80 ± 0.09 polyps). It was also 20.7% larger on PVC (3.38 ± 0.13 polyps) than on PC (2.80 ± 0.09 polyps).

## 3.3 New settlement after 21 months

One year after the 2021 analysis (after 21 months in situ), the same tiles were retrieved. 1490 new settlers were counted on the 60 tiles, making an average of 9.8 ± 0.93 new settlers.100 $cm^{-2}$. This was significantly higher than settler density on the same tiles after nine months (5.62 ± 0.57 settlers.100 $cm^{-2}$) (Kruskal-Wallis test, $p < 0.001$). There was a significant effect of tile side on new settler density after 21 months, but this effect was dependent on material and crevices as indicated by the significant interactions side x material and side x crevices (Fig. 6.2A & Table 6.S1B). New settler densities on upper sides were increased by 161.8% on PC (7.41 ± 1.26 settlers.100 $cm^{-2}$) relative to PVC (2.83 ± 0.59 settlers.100 $cm^{-2}$), while no difference between materials occurred on undersides. New settler densities on undersides were increased by 63.7% and 480.9% on PC (12.13 ± 1.63 settlers.100 $cm^{-2}$) and PVC (16.44 ± 2.43 settlers.100 $cm^{-2}$), respectively, relative to upper sides (PC: 7.41 ± 1.26 settlers.100 $cm^{-2}$, PVC: 2.83 ± 0.59 settlers.100 $cm^{-2}$). With regards to crevice addition, new settler densities on upper sides were increased by 27.3% on tiles with crevices (5.73 ± 0.67 settlers.100 $cm^{-2}$) relative to flat tiles (4.50 ± 1.35 settlers.100 $cm^{-2}$), while the opposite occurred on undersides, with a decrease by 60.2% on tiles with crevices (10.98 ± 1.39 settlers.100 $cm^{-2}$) relative to flat tiles (17.59 ± 2.49 settlers.100 $cm^{-2}$). New settler densities on both tiles with and without crevices were significant higher on undersides than on upper sides.

## 3.4 One-year settler survival and growth

Settler survival on undersides was significantly influenced by material (Table 6.S2B). It was 4.7-fold increased on PVC (5.56 ± 2.41%) relative to PC (1.18 ± 0.73%) (Fig. 6.2C). Settler survival

on PC was not influenced by side or crevices (Table 6.S2B). In contrast, settler survival on tiles with crevices was significantly influenced by side and material (Table 6.S2B). Settler survival on tiles with crevices was 3-fold increased on upper sides (15.01 ± 3.18%) relative to undersides (4.75 ± 2.37%), and 8-fold increased on PVC (16.02 ± 3.27%) relative to PC (1.93 ± 1.07%). For settler growth, we were only able to detect an effect of crevice addition on the growth of settlers present on undersides, as well as on PC (Table 6.S2C). Settler growth on undersides was 4-fold increased on tiles with crevices (10.70 ± 2.17) relative to flat surfaces (2.27 ± 0.66) (Fig. 6.2D). Settler growth on PC was 4.8-fold increased on tiles with crevices (11.73 ± 3.49) relative to flat surfaces (2.44 ± 0.85).

## 3.5 Preferred tile areas on tiles with crevices

There were significant interactions between side x tile area and material x tile area on settler density on tiles with crevices (Table 6.S3A). On the upper sides, settler densities were 3.7-fold increased inside crevices of 12x12x12 mm (5.27 ± 0.84 settlers.100 $cm^{-2}$) relative to the exposed area of the tiles (1.42 ± 0.42 settlers.100 $cm^{-2}$) (Fig. 6.3A). Other crevices did not significantly differ from the exposed area. In contrast, settler densities on undersides were 4-fold increased on the exposed area (6.88 ± 1.01 settlers.100 $cm^{-2}$) relative to crevices (1.71 ± 0.39 settlers.100 $cm^{-2}$). Settler densities on PC tiles were 2.8-fold increased on exposed area (5.31 ± 0.96 settlers.100 $cm^{-2}$) relative to crevices (1.87 ± 0.39 settlers.100 $cm^{-2}$). On PVC tiles, settler densities did not differ between tile areas when looking at both sides; however, separate posthoc tests on the upper sides revealed that settler densities were significantly higher in all crevice sizes relative to the exposed area (Fig. 6.3A).

Similar trends were found for the new settlement observed on 21-month-old tiles (Table 6.S3B; Fig. 6.3B). However, the 3-way interaction side x material x tile area was significant. On upper PVC tiles, settler densities were 117.8-fold increased inside all squared crevices (9.42 ± 1.24 settlers.100 $cm^{-2}$) relative to the exposed area (0.08 ± 0.08 settlers.100 $cm^{-2}$). On the upper side of PC tiles, settler densities were significantly higher on the exposed area and inside the two largest crevices (5.63 ± 0.82 settlers.100 $cm^{-2}$) relative to the small elongated 2x3x40 mm crevices (0 settlers.100 $cm^{-2}$). On the underside of PVC tiles, settler densities were 12.8-fold increased on the exposed area (17.03 ± 4.76 settlers.100 $cm^{-2}$) relative to the largest crevice (1.33 ± 0.91 settlers.100 $cm^{-2}$). On the underside of PC tiles, settler densities were significantly higher on the exposed area (15.78 ± 3.02 settlers.100 $cm^{-2}$) relative to all crevices, which had all less than 1.6 settlers.100 $cm^{-2}$.

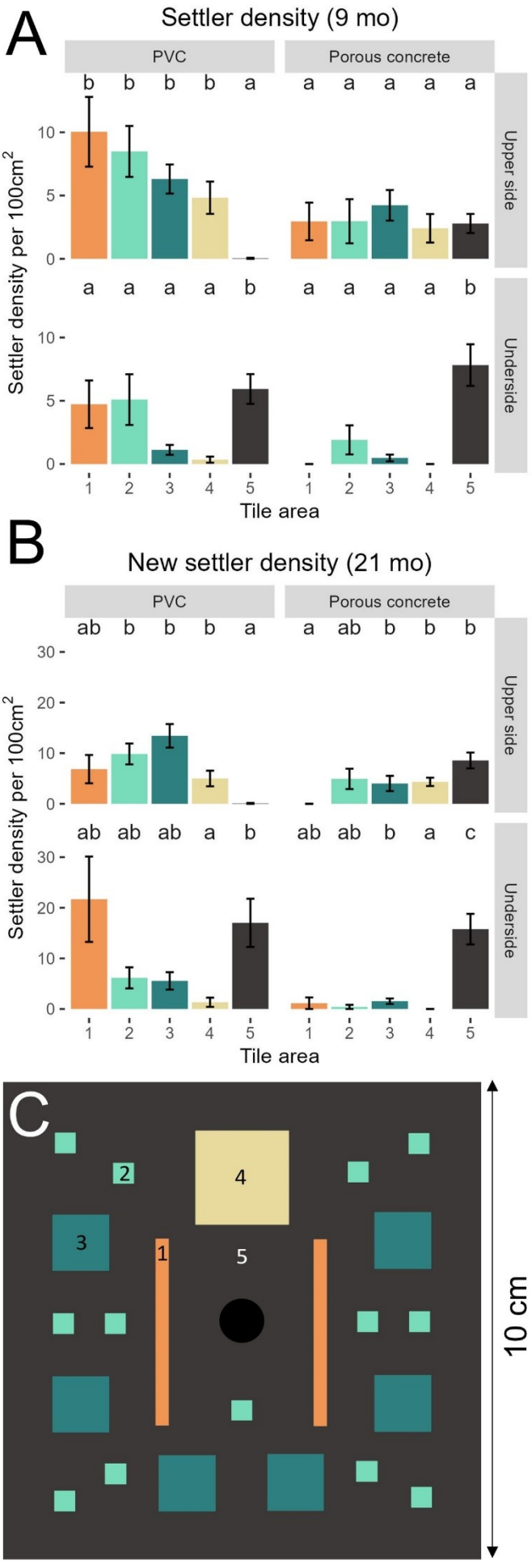



**Figure 6.3.** Density (mean ± SEM) of coral recruits (after 9 months in situ, 2020 and 2021) for each tile area, on each material and side. Letters represent significantly different groups (A). Density of new coral recruits (after 21 months in situ, 2022, without 2021 survivors) (B). Representation of different tile areas: 1 = crevice of 40x3x2 mm, 2 = crevice of 5x5x5 mm, 3 = crevices of 12x12x12 mm, 4 = crevices of 20x20x20 mm, 5 = rest of flat surface (8025 mm$^2$) (C).

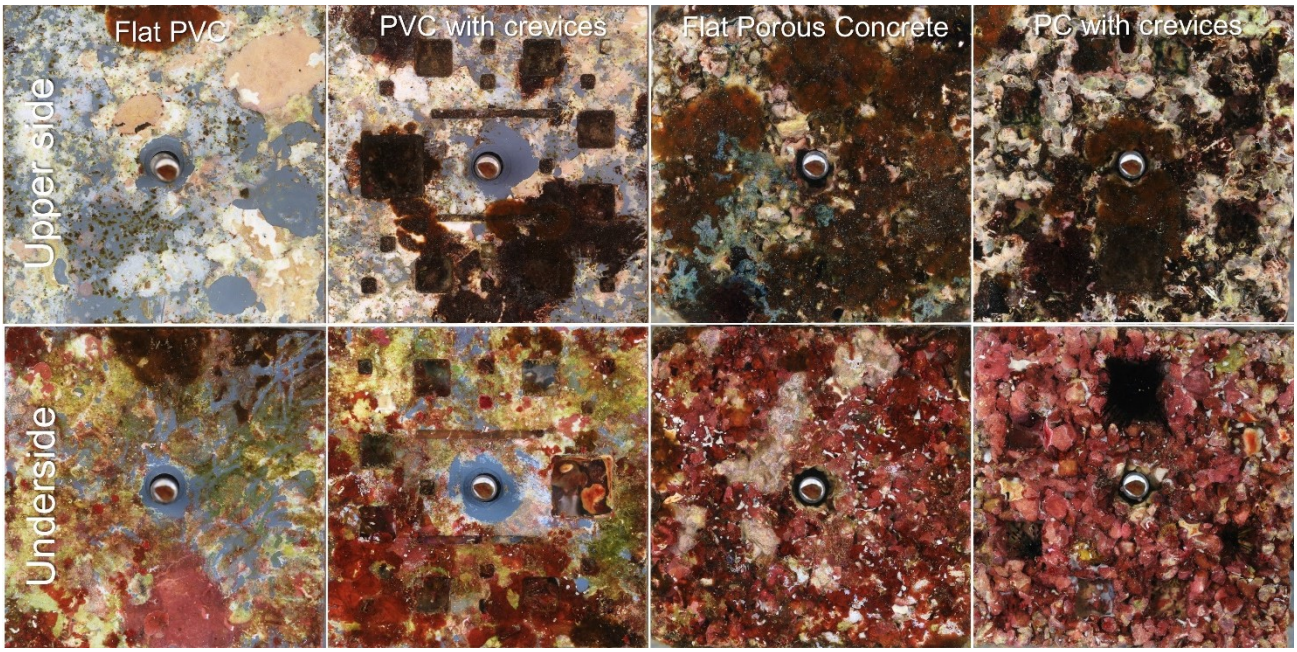



**Figure 6.4.** Photos of settlement tiles nine months after deployment showing the upper and undersides of each tested material (PVC and Porous concrete) with and without added crevices.

## 3.6 Benthic composition

After nine months of immersion in situ, settlement tiles were covered by diverse benthic communities (Fig. 6.4). Benthic composition on the tiles after nine months did not differ between 2020 and 2021 (Adonis2 Permanova p-value = 0.189). All factors significantly interacted to influence the benthos (manyglm: Dev 23.0, p = 0.014; Table 6.S4). However, tile side had the largest influence (Dev 510.3, p < 0.001), followed by material (Dev 291.1, p < 0.001), then to a lower extent the interaction side x material (Dev 78.1, p < 0.001) and crevices (Dev 71.7, p < 0.001). Globally, the upper sides of PC tiles were discriminated by their abundant cover of *Lobophora* (38.18 ± 2.95%) and live CCA (35.19 ± 2.48%), while the undersides of PC tiles were discriminated by their high cover of bryozoans (5.45 ± 0.89%), ERA (29.80 ± 1.81%) and sponges (7.18 ± 0.71%) (Fig. 6.5). In contrast, the upper sides of PVC tiles were dominated by live CCA (50.63 ± 2.40%) and bare substrate (19.99 ± 1.64%), while the undersides of PVC tiles were discriminated by their high abundance of dead CCA (16.64 ± 1.35%), other algae (13.37 ± 1.64%) and other fauna (4.01 ± 0.50%). After 21 months of immersion in situ, settlement tiles were visually similar to 9-month-old tiles (Fig. 6.S2). When running the model using the tiles immersed in 2021 and adding age (9 vs 21 months) as an additional factor (Table 6.S5), the most influential factors and interactions were the same as the previous analysis without age, suggesting relatively small temporal changes in the benthos. Overall, there were significantly less bryozoans, sponges, dead CCA, and other algae and more live CCA, ERA and *Lobophora* at 21 months compared with nine months (Kruskal-Wallis test pvalue < 0.05).

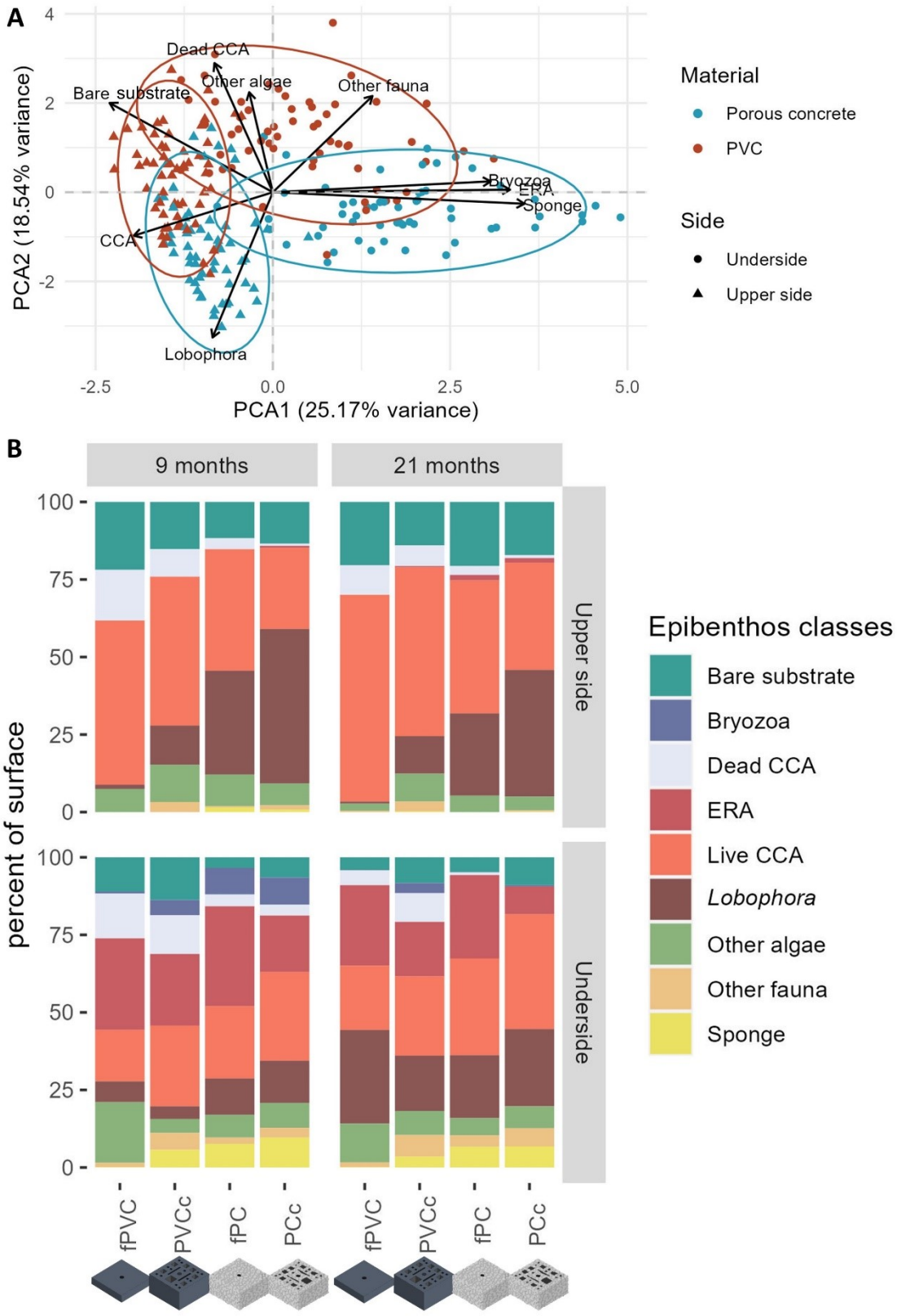



**Figure 6.5.** Principal componant analysis of benthic cover on upper and undersides of PVC and Porous concrete settlement tiles (A). Percent of cover by nine major benthic categories after nine months (2021) and 21 months (2022) at 10 meters on the forereef of Mo'orea (B), on tiles of different materials with and without crevices: fPVC = flat Polyvinyl Chloride, PVCc = PVC with crevices, fPC = flat porous concrete, PCc = PC with crevices.

## 3.7 Influence of the benthos on coral settlement and settler survival

There were relatively few significant correlations between coral settlement and benthic cover after 9 and 21 months (Table 6.S6, Fig. 6.6). On the upper sides, other algae were positively correlated with settler density after nine months in 2020. New settlement in 2022 correlated positively with *Lobophora* and encrusting red algae, and negatively with live and dead CCA. On the undersides, live CCA was positively correlated with settler density after nine months in 2020. Settler survival on the upper sides was on the other hand negatively correlated with *Lobophora*, and positively with live CCA and other fauna. On the undersides, survival was negatively correlated with other algae.

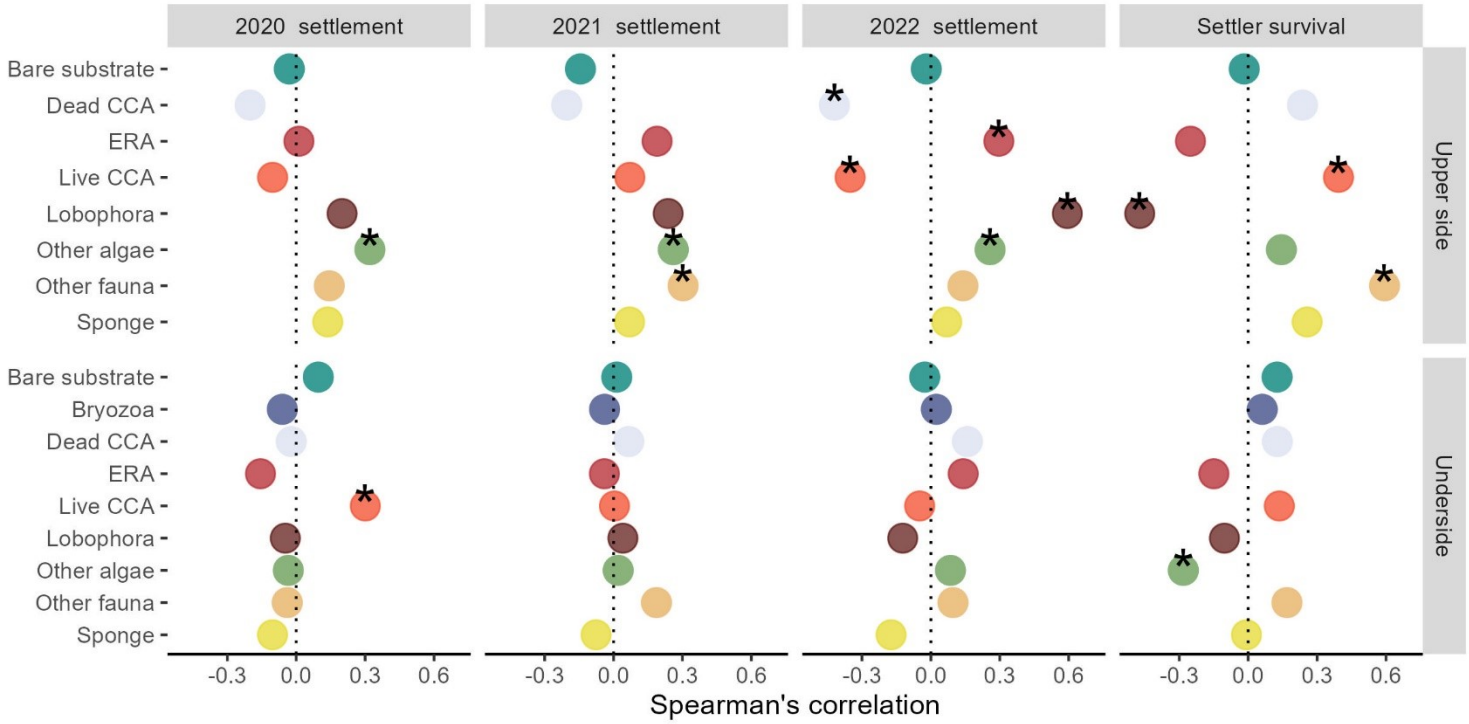



**Figure 6.6.** Spearman's correlation index between all benthic categories and either density of coral settlers (in 2020, 2021 and 2022) or settler survival, on upper and undersides. * represent significant correlations.

## 4. Discussion

Our study aimed to investigate how porosity and the addition of crevices influenced natural coral recruitment on artificial materials. Settler density averaged 3.86 settlers.100 $cm^{-2}$ after 9 months and 9.8 settlers.100 $cm^{-2}$ after 21 months, with only 6.1% settler survival between 9 and 21 months. The addition of crevices significantly interacted with material and side to influence settler density. Tiles with added crevices were more efficient on upper sides, or on PVC. While PVC was less successful than PC in terms of settler density, it performed better in terms of settler size and survival with added crevices. Similarly to previous studies in Mo'orea, Pocilloporidae, followed by Acroporidae, represented the large majority of settlers counted on settlement tiles (Price 2010; Leonard et al. 2022). The very small percentage of Poritidae and other families could be explained by the low abundance of these families on the forereef of Mo'orea. Since the crown of thorn invasion and cyclone of 2010, Mo'orea's reefs have recovered their coral cover, but with a large dominance of Pocilloporidae (Adjeroud et al. 2018; Pérez-Rosales et al. 2021a).

The efficiency of different complexities as settlement substates are coherent across the two tested periods (2020 and 2021). However, 2021 showed a much higher coral settlement than 2020 (2.8-fold increase). This could be the 2019 bleaching event reducing the reproductive potential of surviving colonies. In fact, French Polynesia experienced a widespread and prolonged bleaching event in April 2019 (Leinbach et al. 2021). In Mo'orea, this event led to up to 72% of Pocilloporid colonies being bleached, and up to 42% mortality around the island (Burgess et al. 2021). Prolonged thermal stress can cause a drop in the fecundity of coral colonies (Burt & Bauman 2019), and a significant reduction in sperm motility (Omori et al. 2001). This could have decreased the reproductive output by the end of 2019 and could explain the low settlement observed in 2020, similar to what has been observed in different sites around Mo'orea (Edmunds et al. 2024). Temperatures were also abnormally high in the austral summer 2020 (March-April) compared to 2021, which could have led to a higher mortality among the young settlers before the tiles were retrieved in May (Fourney & Figueiredo 2017).

The side of the settlement tiles had a significant effect on settler density and interacted with the addition of crevices. Larvae preferentially chose to settle on the undersides of the tiles devoid of any surface complexity, which is consistent with other studies (Birkeland 1977; Maida et al. 1994; Adjeroud et al. 2007). On tiles with added crevices or on porous concrete, settlement after nine months was similar on upper and undersides. In fact, no settlers were observed on upper flat PVC surfaces, and all settlers were within the crevices of PVC, similarly to what can be observed on exposed ceramic tiles (Edmunds et al. 2014; McDevitt-Irwin et al. 2023). Larvae may sense light that directs them preferentially towards cryptic and shaded habitats (Edmunds et al. 2004). On porous concrete, settler density was higher than on PVC, but the addition of crevices did not further increase settlement success. Thus, the most complex material does not yield the highest settler densities, and increasing surface complexity enhances recruitment up to a certain threshold. While settler densities were higher on undersides, settlers grew and survived better on upper sides. Birkeland also observed a

faster growth on upper surfaces of rough concrete blocks (Birkeland 1977). However, in other studies, survival was low on upper surfaces of tiles, even when these were grooved (Randall et al. 2021). While settlers were more abundant on PC, they grew and survived better on PVC. Survival rates on PC and undersides were lower than on upper PVC with added crevices.

Crevices need to have a certain size to effectively promote coral settlement and survival. Coral larvae are about 1 mm when they are competent to settle (Schmidt-Roach et al. 2008), thus holes between 1 and 5 mm could be suitable to protect them from predators. Whalan et al. found that coral larvae preferred small 0.4 mm crevices compared to larger ones (0.7-1 mm) (Whalan et al. 2015). However, the benefits of very small crevices may shift with settler age. We found that long and narrow crevices (2x3x40 mm) were less used than squared crevices, between 5- and 20-mm in width and depth. Doropoulos et al. also found the highest coral settler density in natural crevices that were around 10 mm wide and 10 mm deep (Doropoulos et al. 2016). Randall et al. found that initial settlement was higher in 2 mm wide gaps than 4 mm wide gaps, but survival subsequently increased in larger and deeper crevices (Randall et al. 2021). While crevices increased survival in *Pocillopora* and *Porites* micro-fragments, the actual crevice size (either squares of 12x12x10 mm or 20x20x20 mm) had no effect in a previous study (Gallagher & Doropoulos 2017), similarly to our findings. In Japan, narrow gaps of 5 mm lead to high (12.4%) survival in coral settlers compared to large shallow gaps (15 or 25 mm wide) even after two years (Nozawa 2012). Thus Nozawa suggests gaps of less than 10 mm wide and over 2 mm deep to enhance survival (Nozawa 2012). Bigger holes might protect them from scrapers such as parrotfish, but not corallivorous species that have a fine mouth and can target settlers larger than 3 mm (Doropoulos et al. 2016). There were also more settlers between medium and coarse mesh (5 and 7 mm wide) than on fine mesh (4 mm) (Brock 1979). Taking into account these results together with our findings, we suggest that crevices deeper and larger than 5 mm, on a smooth material, can increase settlement and survival rates. Larger refuges will benefit corals by allowing them to reach larger sizes. Once they reach a certain size-escape threshold, coral recruits become less subject to predation, and their survival chance consequently increases (Raymundo & Maypa 2004; Doropoulos et al. 2012).

A longer conditioning of artificial materials (21 versus 9 months) also seemed to increase coral settler density. This could be due to an even higher reproductive output during the spawning season at the end of 2021, as natural coral recruitment can be highly variable in different years (Adjeroud et al. 2007). However, we did not measure the base settler density on clean tiles after 9 months in 2022. A positive impact of a longer immersion time could be explained by the attraction of new larvae by the surviving settlers already on the tiles (Dixson et al. 2014; Da-Anoy et al. 2017, 2020). or by an As the benthos ages, coral larvae can change their settlement choice and settle preferentially on CCA (Elmer et al. 2018). Between 9 and 21 months, the major changes observed were an overall increase in live CCA, ERA and *Lobophora* and a decrease in sponges, bryozoans and dead CCA. There was however a negative correlation between the abundance of dead and live CCA and coral settlement after 21 months, but only

on upper sides. Upper sides harbored high CCA cover, similarly to other studies (Evensen et al. 2021). Both live and dead CCA patches, were abundant on upper flat PVC, which hosted almost no settlers as it offered no surface complexity. CCA species living on exposed surfaces are usually not chosen as settlement substrates by coral larvae (Jorissen et al. 2021). Cryptic CCA species on the other hand, can enhance coral recruitment (Jorissen et al. 2021), which can explain the positive correlation between settler density and CCA cover on the undersides. Depending on the species, CCA are known to enhance coral settlement (Morse et al. 1994; O'Leary et al. 2012) and coral survival, but they can also act as competitors (Jorissen et al. 2020). When settling onto CCA, coral settlers can be removed by the shedding of the CCA's outer layers (Harrington et al. 2004). CCA can also easily overgrow young coral settlers (Harrington et al. 2004; Price 2010; Jorissen et al. 2020). This negative link was also observed on ceramic tiles (Elmer et al. 2016) and on vertical aragonite plugs (Page et al. 2024). In our study, the presence of live CCA on upper sides was positively linked to settler survival after 21 months, possibly because the abundance of CCA lowered overgrowth of coral settlers by turf algae (Jorissen et al. 2020).

*Lobophora* was also very abundant on upper sides, especially on porous concrete. It was positively correlated with settler densities after 21 months but negatively with settler survival. Typically, *Lobophora* has negative impacts on coral microbiome, settlement and settler survival (Morrow et al. 2017; Evensen et al. 2019a, 2019b). It causes bleaching of coral tissue through allelopathic chemicals (Rasher & Hay 2010; Vieira et al. 2016), can also cause larval mortality and decrease settlement (Fong et al. 2019; Page et al. 2023), and is known to overgrow corals (Eich et al. 2019). Coral larvae might initially be attracted to habitats that are also favorable to *Lobophora*, in our case porous concrete, but might later show very low survival due to the competition with this macroalgae. Thus, settler density does not indicate the adequacy of a settlement material for long-term settler survival.

The presence of encrusting red algae, other fauna and other algae was also positively correlated with settler density, and other fauna was positively linked to settler density and settler survival on upper sides. However, because the coverages of these groups were very low on upper sides (< 3.3%), these correlations should be taken with caution. Encrusting red algae, mostly composed of peyssonnelid algal crusts (Edmunds et al. 2023), show varying effects on corals. They can be positively linked with the presence of coral recruits (Elmer et al. 2016). For example, *Peyssonnelia rubra* does not hinder coral settlement or growth, and even enhance coral survival (Maypa & Raymundo 2004). On the other hand, other Peyssonneliaceae compete with and overgrow living corals (Pueschel & Saunders 2009; Edmunds et al. 2019), and display a microbiome less favorable to coral larvae than CCA (Wilson et al. 2020).

On undersides, adding surface complexity actually decreased recruitment, and larvae very rarely chose to settle into crevices. Competition with other organisms can shape recruitment dynamics. We observed more sponges, bryozoans and other fauna (tunicates, Vermetid snails, Serpulid worms, bivalves and foraminiferans) on undersides than on upper sides, and more on tiles with added crevices than without. These organisms can compete with coral recruits

(Dunstan & Johnson 1998; Elmer et al. 2016). Sponges can overgrow and partially kill corals (Schönberg & Wilkinson 2001; Chaves-Fonnegra & Zea 2011), and typically colonize space previously occupied by corals (González-Murcia et al. 2023). Some can even reduce natural coral recruitment (Brandt et al. 2019). However, no significant correlations were observed with settler density on undersides in our study, except for a positive link with CCA cover. In addition, other algae (cyanobacteria, turf and macroalgae other than *Lobophora*) negatively impacted settler survival. In the absence of grazing, turf algae can negatively impact settler survival (Doropoulos et al. 2017). The fact that survival was very low on porous concrete and on undersides suggests that coral settler predation is not the main factor driving settler survival. Indeed, when protected from predation on undersides and within pores and crevices of porous concrete, settlers still had very low survival rates (3.4% after a year). In accordance with previous studies, competition with algae and invertebrates may be the main driver of coral mortality in ungrazed areas (Doropoulos et al. 2016). This could explain the low survival on undersides. Survival on undersides was, however, significantly increased on PVC relative to PC. Thus, while PVC might not be optimal for initial coral settlement, it might attract less competitors than porous concrete and favor survival. No larvae settled within the largest crevices on PC undersides. Light availability could also limit recruitment within crevices on undersides and within too deep crevices on upper sides (Maida et al. 1994).

In summary, we conclude that adding crevices or porosity can enhance coral settler density on light-exposed surfaces. However, porous materials can lead to the colonization of unwanted benthic organisms and decrease settler survival. The inclusion of shelters adapted to coral settlers, in the form of crevices, should be a priority when designing artificial reefs, but not on the undersides, which are already sheltered and received less light. Crevices should be between 0.5 and 2 cm in width and depth to optimize settler growth and survival. Finetuning the design of artificial reefs could improve their success in attracting and promoting the survival of coral settlers, thus enhancing the biodiversity and productivity of degraded reefs.

## Acknowledgments

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## 5. Supplementary material

**Table 6.S1.** Anova results for generalized mixed models explaining each variable influencing coral settler density after 9 months and after 21 months

| Response variable | Explanatory factor | $X^2$ | df | p-value | Pairwise | p-value (KW) |
|---|---|---|---|---|---|---|
| **A. Settler density (after 9 months)** | **Year** | **28.39** | **1** | **< 0.001** | **2021 > 2020** | |
| | **Side** | **14.96** | **1** | **< 0.001** | **Under > Upper** | |
| | **Material** | **5.71** | **1** | **0.017** | **PC > PVC** | |
| | Crevices | 1.63 | 1 | 0.202 | | |
| | Side*Material | 1.77 | 1 | 0.184 | | |
| | **Side*Crevices** | **9.27** | 1 | **0.002** | **Upper: Crevices > Flat** | **< 0.001** |
| | | | | | **Under: Flat > Crevices** | **0.005** |
| | | | | | Crevices: Upper = Under | 0.273 |
| | | | | | **Flat: Under > Upper** | **< 0.001** |
| | **Material*Crevices** | **5.63** | 1 | **0.018** | **PVC: Crevices > Flat** | **0.004** |
| | | | | | PC: Crevices = Flat | 0.105 |
| | | | | | Crevices: PVC = PC | 0.324 |
| | | | | | **Flat: PC > PVC** | **0.001** |
| | Side*Material* Crevices | 3.60 | 1 | 0.058 | | |
| **B. New settlement (after 21 months)** | **Side** | **37.03** | **1** | **< 0.001** | **Under > Upper** | |
| | Material | 0.01 | 1 | 0.928 | | |
| | Crevices | 3.18 | 1 | 0.075 | | |
| | **Side * Material** | **8.69** | **1** | **0.003** | **Upper: PC > PVC** | **< 0.001** |
| | | | | | Under: PC = PVC | 0.255 |
| | | | | | **PC: Under > Upper** | **0.012** |
| | | | | | **PVC: Under > Upper** | **< 0.001** |
| | **Side * Crevices** | **6.76** | **1** | **0.009** | **Upper: Crevices > Flat** | **0.007** |
| | | | | | **Under: Flat > Crevices** | **0.045** |
| | | | | | **Crevices: Under >** | **< 0.001** |
| | | | | | **Upper** | **< 0.001** |
| | | | | | **Flat: Under > Upper** | |
| | Material * Crevices | 1.75 | 1 | 0.186 | | |
| | Side * Material * Crevices | 1.34 | 1 | 0.246 | | |

**Table 6.S2.** Anova results for generalized mixed models explaining each variable influencing settler survival and growth

| Fixed: | Underside | | | | | Porous concrete | | | | | Tiles with crevices | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Respo-nse var.** | **Explanato-ry factor** | **$X^2$** | **df** | **p-val** | **Pairwise** | **Explanato-ry factor** | **$X^2$** | **df** | **p-val** | **Pairwise** | **Explanato-ry factor** | **$X^2$** | **df** | **p-val** | **Pairwise** |
| **A. Settler size** | Year | 0.14 | 1 | 0.707 | | Year | 0.08 | 1 | 0.778 | | Year | 0.12 | 1 | 0.734 | |
| | **Material** | **17.20** | **1** | **< 0.001** | **PVC > PC** | **Side** | **24.83** | **1** | **< 0.001** | **Upper > Under** | **Side** | **30.18** | **1** | **< 0.001** | **Upper > Under** |
| | Crevices | 2.47 | 1 | 0.116 | | **Crevices** | **8.45** | **1** | **0.004** | **Crevices > Flat** | **Material** | **9.69** | **1** | **0.002** | **PVC > PC** |
| | Material* Crevices | 0.78 | 1 | 0.377 | | **Side*Crevices** | **13.59** | **1** | **< 0.001** | **Upper: Text > Flat,** Under: no dif **Crevices: Upper > Under,** Flat: no dif | Side*Material | 2.70 | 1 | 0.100 | |
| **B. Settler survival** | **Material** | **4.75** | **1** | **0.029** | **PVC > PC** | Side | 0.67 | 1 | 0.413 | | **Side** | **21.79** | **1** | **< 0.001** | **Upper > Under** |
| | Crevices | 0.05 | 1 | 0.469 | | Crevices | 1.67 | 1 | 0.197 | | **Material** | **31.66** | **1** | **< 0.001** | **PVC > PC** |
| | Material* Crevices | 1.57 | 1 | 0.210 | | Side *Crevices | 2.98 | 1 | 0.084 | | Side*Material | 1.73 | 1 | 0.189 | |
| **C. Settler growth** | Material | 0.38 | 1 | 0.538 | | Side | 1.24 | 1 | 0.265 | | Side | 1.82 | 1 | 0.177 | |
| | **Crevices** | **3.95** | **1** | **0.047** | **Crevices > Flat** | **Crevices** | **9.31** | **1** | **0.002** | **Crevices > Flat** | Material | 0.49 | 1 | 0.485 | |
| | Material* Crevices | 0.01 | 1 | 0.932 | | Side *Crevices | 1.07 | 1 | 0.301 | | Side*Material | 0.21 | 1 | 0.650 | |

**Table S3** Anova results for generalized mixed models explaining the influence of different tile areas (1 = crevices of 40x3x2 mm, 2 = crevices of 5x5x5 mm, 3 = crevices of 12x12x12 mm, 4 = crevices of 20x20x20 mm, 5 = rest of flat surface (8025 $mm^2$)) on each variable on tiles with crevices. The pairwise column shows the significantly different groups. Variables are bold when significant. Tile area are ordered from the highest value to lowest, underlined with the same line when not statistically different.

| Response variable | Explanatory factor | $X^2$ | df | p-value | Pairwise |
|---|---|---|---|---|---|
| A. Recruit density (9 months) | **Year** | **4.76** | **1** | **0.029** | **2021 > 2020** |
| | Side | 0 | 1 | 0.99 | |
| | **Material** | **9.73** | **1** | **0.002** | **PVC > PC** |
| | Tile area | 6.65 | 4 | 0.156 | |
| | Side * Material | 0 | 1 | 0.991 | |
| | **Side * Tile area** | **28.65** | **4** | **<0.001** | **Upper: 3 1 2 4 5**<br>**Under: 5 2 3 1 4**<br>**1-4: Upper > Under**<br>**5: Under > Upper** |
| | **Material * Tile area** | **20.78** | **4** | **<0.001** | **PC: 5 2 3 1 4**<br>PVC: 1 2 3 5 4<br>**1-4: PVC > PC**<br>**5: PC > PVC** |
| | Side * Material * Tile area | 7.73 | 4 | 0.102 | |
| B. New recruitment (21 months) | Side | 0 | 1 | 0.989 | |
| | Material | 0 | 1 | 0.988 | |
| | Tile area | 4.71 | 4 | 0.318 | |
| | Side * Material | 0 | 1 | 0.99 | |
| | **Side * Tile area** | **16.6** | **4** | **0.002** | **Upper: 3 4 2 5 1**<br>**Under: 5 3 1 2 4**<br>**2-4: Upper > Under**<br>**5: Under > Upper** |
| | **Material * Tile area** | **28.34** | **4** | **<0.001** | **PC: 5 3 4 2 1**<br>**PVC: 3 2 5 1 4**<br>**1-3: PVC > PC**<br>**5: PC > PVC** |
| | **Side * Material * Tile area** | **12.05** | **4** | **0.017** | **PVC upper side: 3 2 4 1 5**<br>**PC upper side: 5 4 3 2 1**<br>**PVC underside: 5 1 2 3 4**<br>**PC underside: 5 3 1 2 4** |

**Table 6.S4.** *Anova* results of *manyglm* model on the influence of explanatory variables on the species composition of the benthic cover of settlement tiles (2020 and 2021).

| Explanatory | Res.Df | Df.diff | Dev | Pr(>Dev) |
|---|---|---|---|---|
| Intercept | 231 | | | |
| **Side** | **230** | **1** | **510.2573** | **0.001** |
| **Material** | **229** | **1** | **291.0861** | **0.001** |
| **Crevices** | **228** | **1** | **71.68263** | **0.001** |
| **Side*Material** | **227** | **1** | **78.08844** | **0.001** |
| **Side*Crevices** | **226** | **1** | **36.06101** | **0.001** |
| **Material*Crevices** | **225** | **1** | **62.08829** | **0.001** |
| **Side*Material*Crevices** | **224** | **1** | **22.97539** | **0.007** |

**Table 6.S5.** *Anova* results of *manyglm* model on the influence of explanatory variables on the species composition of the benthic cover of settlement tiles (2021 and 2022). Bold values are significant ($p < 0.05$).

| Explanatory | Res.Df | Df.diff | Dev | Pr(>Dev) |
|---|---|---|---|---|
| Intercept | 239 | | | |
| **Age** | **238** | **1** | **48.7** | **0.001** |
| **Side** | **237** | **1** | **655.4** | **0.001** |
| **Material** | **236** | **1** | **173.5** | **0.001** |
| **Crevices** | **235** | **1** | **92.3** | **0.001** |
| **Age*Side** | **234** | **1** | **61.8** | **0.001** |
| **Age*Material** | **233** | **1** | **45.9** | **0.001** |
| **Side*Material** | **232** | **1** | **99.9** | **0.001** |
| Age*Crevices | 231 | 1 | 11.0 | 0.339 |
| **Side*Crevices** | **230** | **1** | **89.8** | **0.001** |
| **Material*Crevices** | **229** | **1** | **76.7** | **0.001** |
| **Age*Side*Material** | **228** | **1** | **30.4** | **0.001** |
| Age*Side*Crevices | 227 | 1 | 8.8 | 0.422 |
| Age*Material*Crevices | 226 | 1 | 6.8 | 0.700 |
| **Side*Material*Crevices** | **225** | **1** | **43.2** | **0.001** |
| Age*Side*Material*Crevices | 224 | 1 | 11.4 | 0.125 |

**Table 6.S6.** Spearman's correlation test between benthic categories and coral settlement at 9 months (2020, 2021) and new settlement at 21 months (2022), and settler survival, on upper and undersides. Bold values are significant ($p < 0.05$).

| | | 2020 settlement | | 2021 settlement | | 2022 settlement | | Settler survival | |
|---|---|---|---|---|---|---|---|---|---|
| **Side** | **Benthos** | **Rho** | **P-value** | **Rho** | **P-value** | **Rho** | **P-value** | **Rho** | **P-value** |
| **Upper side** | Bare Substrate | -0.030 | 0.826 | -0.146 | 0.267 | -0.020 | 0.880 | -0.017 | 0.919 |
| | **Live CCA** | -0.103 | 0.451 | 0.075 | 0.567 | **-0.351** | **0.006** | **0.393** | **0.016** |
| | **Dead CCA** | -0.201 | 0.138 | -0.213 | 0.102 | **-0.420** | **0.001** | 0.236 | 0.159 |
| | **Encrusting red algae** | 0.012 | 0.932 | 0.180 | 0.168 | **0.296** | **0.022** | -0.251 | 0.133 |
| | **Lobophora** | 0.200 | 0.140 | 0.238 | 0.067 | **0.594** | **0.000** | **-0.472** | **0.003** |
| | **Other algae** | **0.321** | **0.016** | 0.252 | 0.052 | **0.258** | **0.047** | 0.145 | 0.393 |
| | **Other fauna** | 0.144 | 0.290 | **0.313** | **0.015** | 0.140 | 0.287 | **0.593** | **0.000** |
| | Sponge | 0.137 | 0.313 | 0.086 | 0.514 | 0.069 | 0.601 | 0.257 | 0.125 |
| **Underside** | Bryozoa | -0.061 | 0.658 | -0.039 | 0.767 | 0.024 | 0.857 | 0.063 | 0.634 |
| | Bare Substrate | 0.096 | 0.481 | 0.014 | 0.917 | -0.027 | 0.840 | 0.127 | 0.337 |
| | **Live CCA** | **0.301** | **0.024** | 0.004 | 0.976 | -0.049 | 0.710 | 0.136 | 0.304 |
| | Dead CCA | -0.022 | 0.875 | 0.066 | 0.615 | 0.159 | 0.225 | 0.129 | 0.330 |
| | Encrusting red algae | -0.156 | 0.252 | -0.040 | 0.762 | 0.141 | 0.284 | -0.151 | 0.254 |
| | Lobophora | -0.047 | 0.728 | 0.041 | 0.758 | -0.123 | 0.351 | -0.106 | 0.426 |
| | **Other algae** | -0.035 | 0.801 | 0.020 | 0.878 | 0.085 | 0.517 | **-0.284** | **0.029** |
| | Other fauna | -0.039 | 0.775 | 0.187 | 0.153 | 0.096 | 0.464 | 0.168 | 0.202 |
| | Sponge | -0.103 | 0.449 | -0.076 | 0.563 | -0.173 | 0.186 | -0.006 | 0.965 |

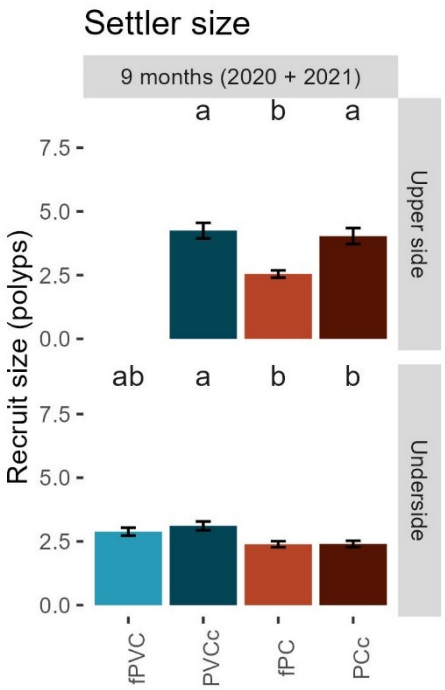



**Figure 6.S1.** Mean (± SEM) size of coral settlers (number of polyps) after 9 months in situ. Letters indicate significantly different groups (Wilcoxon test)

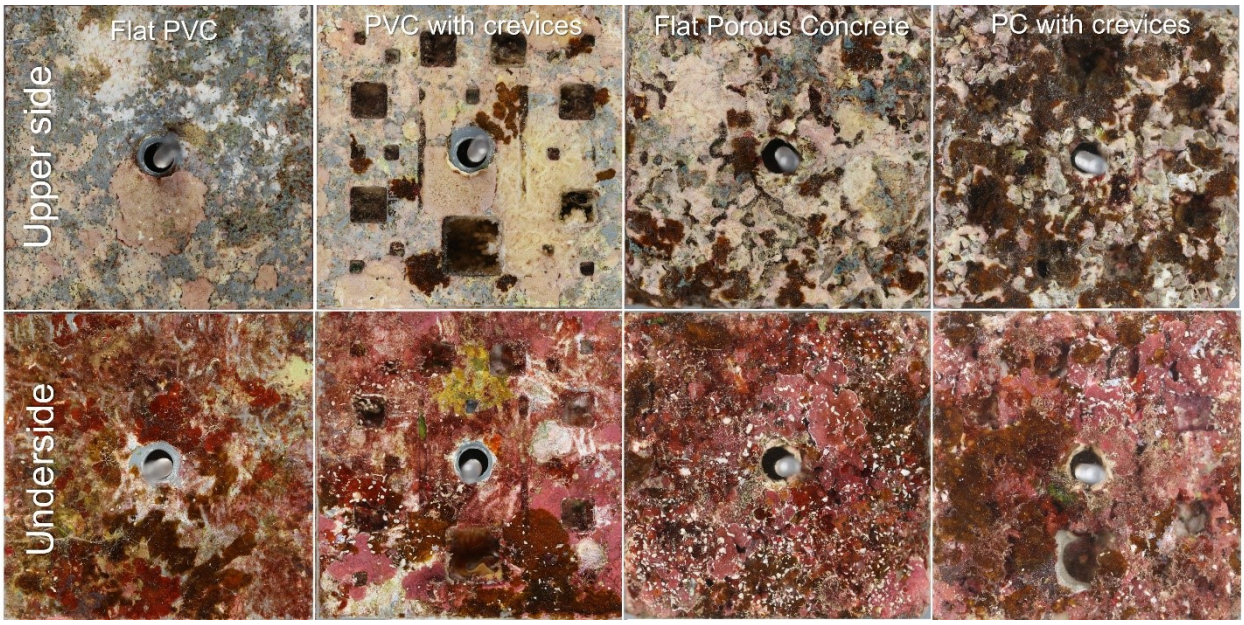
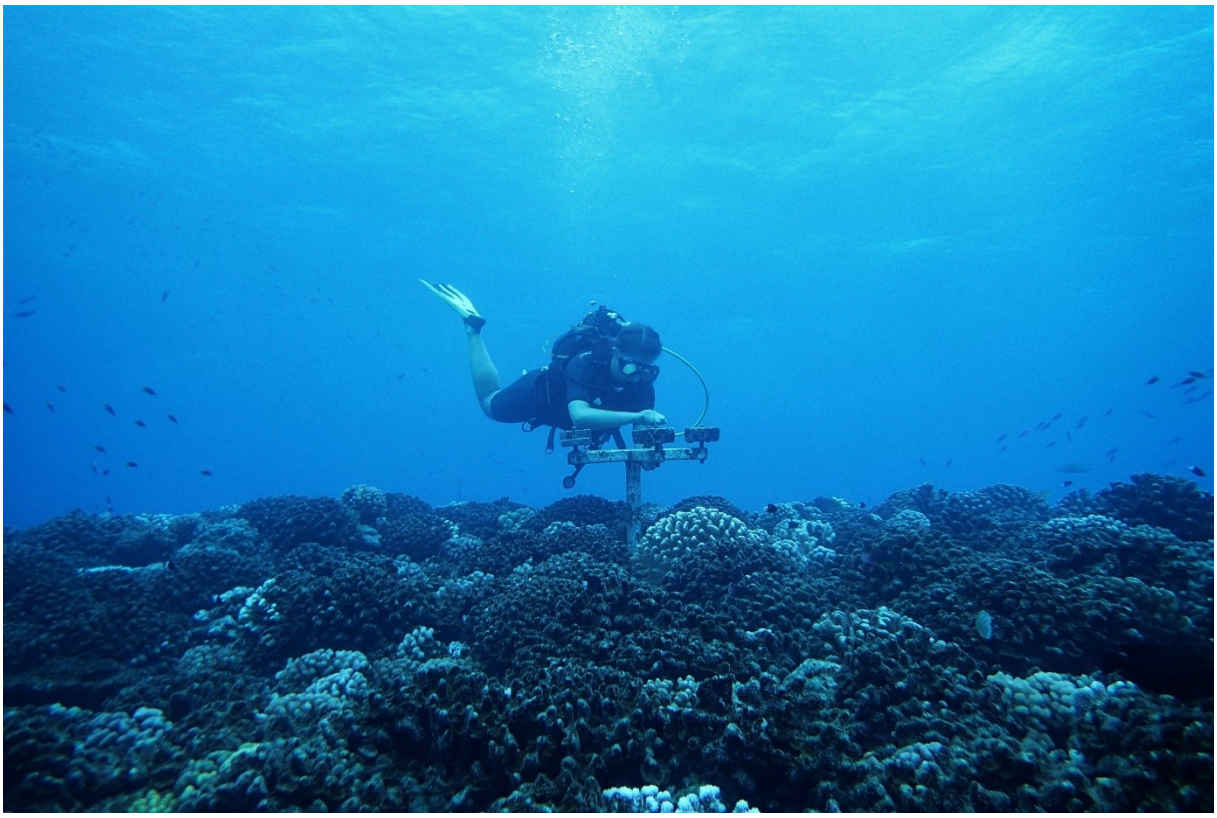



**Figure 6.S2.** Photos of settlement tiles 21 months after deployment, upper and undersides of each tested material (PVC and Porous concrete), with and without crevices.

# Chapter 7

## Coral settlement success on artificial materials is not synonymous with long-term recruit survival



# Coral settlement success on artificial materials is not synonymous with long-term recruit survival

**Running head:** Long-term success of artificial materials

*Camille Leonard[1], Laetitia Hédouin[1,2], Julie Stojakovic[1], Isabelle Davezies[1], Julien Dalle[3], Matthieu Lapinski[3], Philippe Blanc[4], Maggy M. Nugues[2,6]*

[1] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, BP 1013, 98729 Papetoai, Mo'orea, French Polynesia

[2] Laboratoire d'Excellence "CORAIL", Papetoai, Mo'orea, French Polynesia

[3] Seaboost, 889 rue de la Vieille Poste 34000 Montpellier, France

[4] TotalEnergies, Avenue Larribau, 64018 Pau Cedex, France

[5] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan, 52 Avenue Paul Alduy, 66860 Perpignan Cedex, France

## Abstract

With the growing disturbances threatening corals and their recruitment rates, substrate enhancement through artificial reefs is viewed as a palliative technique that could improve coral cover. A better understanding of the influence of material properties on the early life stages of corals is essential for optimized artificial reef design. The present study focuses on the performance of several materials as long-term recruitment substrates. After 18 months, Portland, 3D-printed concretes and PVC displayed the highest settler density, but glass and fiberglass showed the best survival rates. Porous and brittle materials showed very low settler density and survival, suggesting the porosity and durability of materials significantly impact their performance. The cover of *Lobophora* and turf algae was positively correlated with settler density, but no benthic category showed significant correlations with settler survival except coral cover. Through significant interactions with time, the influence of material on settler density and survival changed over time, underlining the importance of further long-term monitoring in evaluating the success of recruitment substrates. Encouragingly, the settler density in 2021 was increased almost 3-fold compared to 2020, showing a rapid recovery of normal settlement rates after the 2019 bleaching event. These results highlight the trade-offs displayed by artificial materials in terms of coral density, survival, growth and cover of fouling organisms. Depending on the aims of an artificial reef, the characteristics of selected building materials should be carefully considered beforehand.

# 1. Introduction

Tropical coral reefs are invaluable ecosystems that are increasingly threatened by local and global stressors. Heat waves lead to massive mortalities of reef-building corals (Hughes et al. 2017a), and a significant decrease in their reproductive capacity (Levitan et al. 2014; Hagedorn et al. 2016; Burt & Bauman 2019). Local threats such as urban expansion, overfishing, pollution, dynamite fishing and intensive anchoring lead to vast coral degradation (Edinger et al. 1998; Hughes et al. 2013). Together these stressors lead to widespread shifts towards algal-dominated reefs (Edinger et al. 1998; Hughes et al. 2007; Souter et al. 2021). Between 2009 and 2018, algal cover on coral reefs has increased worldwide by about 20% (Souter et al. 2021). Macroalgae can hinder coral recruitment, both through allelopathy and physical competition (Birrell et al. 2008; Vermeij et al. 2009; Fong et al. 2019). Between 1974 and 2012, coral recruitment is estimated to have decreased by 82% globally (Price et al. 2019). As a result, natural recruitment alone will not be enough to repopulate reefs to their original diversity.

To rehabilitate degraded reefs, restoration techniques have become increasingly popular and elaborate (Boström-Einarsson et al. 2020a). These include direct transplantation, larval seeding, algal removal, and substratum enhancement using artificial materials (Boström-Einarsson et al. 2020a). Coral larvae need solid substrate to metamorphose, as they cannot successfully recruit on mobile substrate such as fine rubble and sand (Maragos 1993). In recent decades, artificial reefs have been used to increase fish abundance, habitat richness and coral cover (Higgins et al. 2022). They can also increase coral settlement rates by offering new surfaces to colonize (Yanovski & Abelson 2019). Typically, they are formed by massive concrete structures: 60% of artificial reefs use concrete, 12% plastics, 9% metal and only 7% rocks (Vivier et al. 2021). Biogenic materials such as shells or coral skeletons are very rarely used for building artificial reefs (Bracho-Villavicencio et al. 2023). However, they have the advantage of blending in with the initial reef and not introducing harmful materials into the environment.

While the first settlement studies deployed slices of coral skeleton (Harriott 1983), various artificial materials have since been used as settlement substrates. Most settlement studies use unglazed ceramic tiles, which are widely available and attract many coral settlers (Harriott & Fisk 1987). Other settlement studies have tested polyvinyl chloride (PVC) (Mallela et al. 2017), rubber (Reyes & Yap 2001), brick tiles (Field et al. 2007), glass tiles (Lei et al. 2021), concrete (Burt et al. 2009), petri dishes (Harriott & Fisk 1987), aragonite plugs (Rodd et al. 2022), fiberglass (Loh et al. 2006) and metal (Fitzhardinge & Bailey-Brock 1989), and recently even 3D printed ceramic (Berman et al. 2023b, 2023a) and 3D printed polylactic acid (PLA) (Ruhl & Dixson 2019; Randall et al. 2021, 2024). Usually, rough materials are preferred over smooth ones (Mallela et al. 2017; Mallela 2018), and a long conditioning in situ is necessary to develop a favorable biofilm (Petersen et al. 2005). In fact, coral larvae prefer to settle in the presence of crustose coralline algae (CCA)(Morse et al. 1994) and associated biofilm (Erwin et al. 2008). In the absence of biogenic cues, some coral larvae can direct themselves towards inorganic cues such as $CaCO_3$, while avoiding other artificial materials such as calcium phosphate, acrylic, and polydimethylsiloxane (PDMS) (Levenstein et al. 2022).

While chemical cues strongly impact the settling behavior of coral larvae, the texture and durability of an artificial material in seawater can influence coral recruitment success (Harrington et al. 2004; Burt et al. 2009). However, few studies have investigated the durability of artificial materials and their ability to sustain long-term coral recruit survival (> one year). In natural conditions, recruit survival is very low, especially in the first weeks after settling (Martinez & Abelson 2013). In only two months, mortality can vary between 49-87% on concrete, rubber or metal tires (Fitzhardinge & Bailey-Brock 1989). On horizontal flat PVC tiles, survival of seeded settlers was on average 12% after one year (Randall et al. 2021). Survival can depend on the coral family or on the site of deployment. For instance, *Pocillopora* nubbins showed much lower survival than *Porites* on exposed concrete tiles (Gallagher & Doropoulos 2017). On concrete tetrapods, 9.6% of *Favia fragum* settlers survived after one year in Curaçao (Chamberland et al. 2017). On the Great Barrier Reef, between 3.5-11.3% *Acropora tenuis* settlers survived after 7 months (Randall et al. 2023). The causes of mortality in coral settlers are diverse. Sedimentation can strongly impact settler survival, especially in the first month (Moeller et al. 2017). However, the major parameter influencing settler survival is usually predation (Spieler et al. 2001). As a consequence, shelters or cryptic surfaces on artificial materials are essential for promoting coral settlement (Price 2010).

When artificial materials display the same orientation and provide the same level of shelter, their mm-scale texture could influence the survival of coral settlers by favoring the growth of different fouling organisms that vary in their interactions with coral settlers (Tebben et al. 2014). For example, very smooth waxed surfaces prevent fouling by turf algae and thus increase the survival or coral settlers compared to ceramic tiles (Tebben et al. 2014). Certain artificial materials can also attract more fleshy algae, sponges, tunicates or CCA than others, which can inhibit coral recruitment (Fitzhardinge & Bailey-Brock 1989; Geraldi et al. 2014). For example, while terracotta, limestone, glass, PVC, polycarbonate and porcelain tiles display a comparable cover of CCA, CCA growth is 3-fold enhanced on terracotta relative to limestone (Kennedy et al. 2017). The fouling communities on artificial materials can be significantly different from those on natural substrata and closely linked to material characteristics, but the underlying causes are still not fully understood (Svane & Petersen 2001). In addition, time is likely to exert an important role on coral recruitment. Using terracotta tiles, Arnold and Steneck (2011) found a ‘‘recruitment window’’ for settling corals from approximately 9 to 14 mo. The window may end as organisms inimical to coral settlement and survival colonize artificial materials.

With the growing use of innovative materials or printing techniques in coral restoration, a greater research effort is needed to fully apprehend their influences on coral early life stages. This paper investigates natural coral settlement on tiles made of different materials on the fore reef of Mo’orea, French Polynesia. Our aim was to test the success of different innovative artificial materials and some more commonly used materials on settler density, survival, and growth. Following a first experiment carried out in 2020 (Leonard et al. 2022), this paper is a replicate of the previous setup, with the exchange of one material and the addition of a longer

survey. The monitoring was carried out after 6, 14 and 18 months. We followed the growth of surviving settlers and the subsequent waves of new settlers at different periods of the year. In fact, coral settlement in the South Pacific displays a peak abundance after the spawning season of September-December, thus settler density on artificial tiles was expected to reach a maximum after December, with a majority of Pocilloporidae settlers (Adjeroud et al. 2007). We hypothesized that settler density would peak at 14 months and that settler survival rates would differ between materials. We also assessed benthic community composition and hypothesized that the abundance of competitive organisms such as *Lobophora* sp. and sponges would negatively correlate with settler survival (Chaves-Fonnegra & Zea 2011; Evensen et al. 2019b; Eich et al. 2019).

## 2. Methods

### 2.1 Experimental setup

Experimental setup, tested materials and analysis are detailed in Leonard et al. (2022). Briefly, ten units were set up between 7 and 13 meters on the forereef in Mo'orea, French Polynesia (17°28.795'S 149°51.135'W). Each carried 13 vertical settlement tiles (100 x 100 mm) made of ten different materials. Three served as controls, as they have commonly been investigated in previous studies: Polyvinyl chloride (PVC), Portland concrete and glass. 7 materials had never been tested as settlement substrates before this first experiment (Leonard et al. 2022): 3D printed rough concrete (2 different compositions and thicknesses, 3DL and 3DS), Aquaroche, Ceramic foam, Fiberglass polymer (FGP), Polylactic acid (PLA) with flax fibers and Porous concrete (Fig. 7.S4 for photos of materials before and after immersion). Tiles were pierced through the center, strung on a threaded rod and separated by a 15 mm plastic spacer, creating a cryptic habitat between each tile protected from grazing fish. Only these sheltered vertical square sides, each receiving the same amount of light, were analyzed in the present paper. PVC tiles were arranged at each end of the stacked tiles and the outer, exposed sides of PVC tiles were not assessed. Units with cleaned tiles were deployed on 28 August 2020 for a period of six months. They were removed by scuba diving, then transported by boat in a cooler filled with seawater to the CRIOBE research station. Each side of the tiles was photographed, the benthic communities were identified, and all coral settlers counted. Tiles were kept in seawater flow-through tables for a maximum of three days before being returned to their initial position on the forereef. They were analyzed a second time in the same way in October 2021 (14 months), and a third time in March 2022 (18 months, see timeline Fig. 7.1). The survival and growth of all settlers were evaluated, and new settlers were counted.

### 2.2 Coral settler survey and benthic community characterization

While immersed in seawater in a plastic container, each tile was screened for coral settlers with a NIGHTSEA SFA Light Head blacklight and glasses equipped with yellow filters, under a

Leica EZ4 HD dissecting stereo microscope. This allowed to easily find coral polyps with their green and red fluorescence. Each tile was then screened under white light and stereo microscope to find corals that did not fluoresce. The position, size (number of polyps) and state (dead or alive) of each coral settler was recorded in an Excel spreadsheet and on a virtual layer on the photo of the tile using the software PhotoQuad (Trygonis & Sini 2012). A grid of 49 points was added to the layer of the tile, and each point was assigned to the benthic organism growing right under that point. Identified categories were live crustose coralline algae (CCA), dead CCA, non-coralline encrusting red algae (such as *Peyssonnelia* spp.), turf algae (filamentous algae), filamentous cyanobacteria, macroalgae (foliose, corticated, or articulated calcareous algae), bryozoans, encrusting foraminiferans, sponges, tunicates, bivalve mollusks, wormtubes (both from Annelids and Vermetid mollusks), live coral, dead organic matter, and bare substrate. To facilitate the orientation of the tile during the following analyses, a blob of modeling epoxy was added to a specific corner of each tile in the 6-month survey. This allowed to orient the tiles in the same way as in the first analysis and overlay the PhotoQuad layer to find previous settlers. Every settler that was not recorded before was counted as a new second or third generation settler during the second and third analyses, respectively.

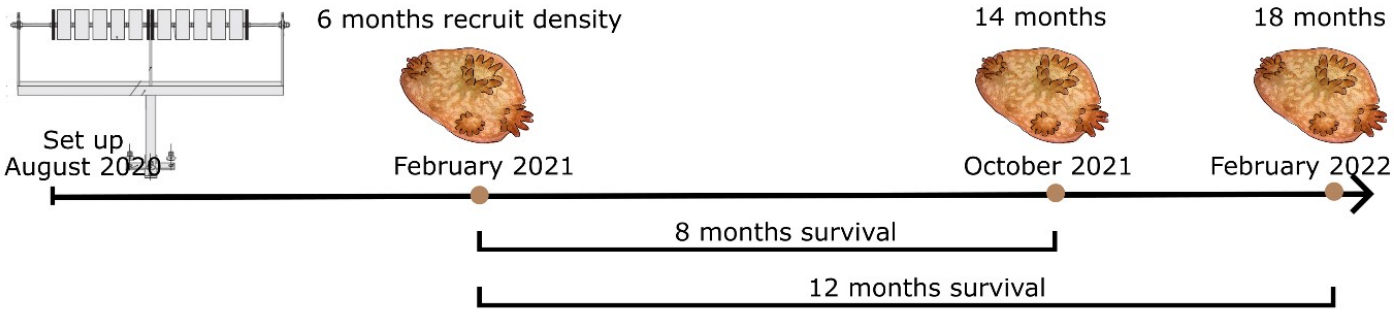



**Figure 7.1.** Timeline of experimental set-up, settler density and survival survey

## 2.3 Statistical analysis

Settler density was expressed as the number of settlers per 100 $cm^2$ and followed a negative binomial distribution. The density of settlers after six months on each material was compared with that of the 2020 data from Leonard et al. (2022). However, one material was different between the 2020 and the present experiment: PVC with chitosan coating was replaced by glass tiles. The effects of material and time on total settler density (sum of new settlers and survivors) was tested using a generalized mixed model with the *glmer* function with a negative binomial distribution, from the *lme4* package (Bates et al. 2015). Material and time were set as fixed factors and unit as a random factor. Main effects and interactions were tested with the *Anova* function from the *car* package (Bates et al. 2007). Pairwise tests were performed with the *emmeans* function of the *emmeans* package (Lenth et al. 2024). The random distribution of the residuals and overdispersion of each model were tested with the *plot*, *qqnorm*, *qqline* and *check_overdispersion* functions of the *performance* package (Lüdecke et al. 2021).

As the second generation of settlers (October 2021-14 months) was much less abundant than the first (February 2021-6 months), only the survival of the first generation after 8 and 12 months was analyzed. The effect of material, time and initial settler density on settler survival was tested with an *Anova* on a generalized mixed model with a *Poisson* distribution, with time, material and initial settler density set as fixed factors, and unit as a random factor. The 4-month survival of new settlers (generation 2, counted after 14 months) and the 4-month survival of the first generation of survivors showed no significant differences on either material, thus were omitted from the results.

Coral settler size distribution did not fit any model, so the size was square root transformed. The effect of material and time on settler survival was tested with an Anova on a generalized mixed model with a negative binomial distribution, with material and time as fixed factors and unit as a random factor.

The growth factor of settlers was calculated by dividing the number of final polyps by the number of initial polyps. The effect of material and time on the growth factor was evaluated by a generalized mixed model with a negative binomial, with material and time as fixed factors and unit as a random factor.

Benthic communities were separated into the 11 most abundant categories (bryozoans, bare surface, live CCA, dead CCA, encrusting red algae (ERA), foraminiferans, *Lobophora* sp., sponges, turf algae, tunicates, worm tubes from annelids and mollusks). The rest were grouped into other fauna (corals, bivalves, anemones…) and other algae (*Dictyota* sp., other macroalgae, cyanobacteria…). Benthic community composition was analyzed using the *mvabund* package (Wang et al. 2012) with the *mvabund* function, *manyglm* with a negative binomial distribution and *anova* function. Material and time were set as fixed factors. To study the significant differences between materials at each time, a pairwise comparison was used on the benthic communities (*mvabund* object) between each material, at each time.

To study the evolution of the benthic cover between 6 and 18 months, the univariate P-values were adjusted for multiple testing, using a step-down resampling procedure, with time (6 and 18 months) as a fixed factor.

Spearman rank-order correlation test (*cor.test* function) was used to examine correlations between benthic categories with settler density at 6 and 18 months and settler survival at 8 and 12 months. Here, the 13 most abundant benthic categories plus “other fauna” and “other algae” were kept, in order to include the influence of coral settlers and bivalves. All data and code used for this study are available on GitHub (https://github.com/CamiLeonard/Artificial_materials).

# 3. Results

## 3.1 Settler density

Settler density after six months averaged 11.20 settlers per 100 $cm^2$ (± 0.68 settlers.100 $cm^{-2}$; mean ± SEM). This was 2.85 times (285%) more than in the same experiment carried out in 2020 (3.93 ± 0.20 settlers.100 $cm^{-2}$) (Leonard et al. 2022). Settler density was significantly impacted by material and immersion time and their interaction (Table 7.S1). Portland concrete had higher settler density (18.1 ± 3.68 settlers.100 $cm^{-2}$) than any other materials, except 3DL concrete (Fig. 7.2A). Glass on the other hand had lower settler densities (1.9 ± 0.64 settlers.100 $cm^{-2}$) than any other tested materials. 3DL concrete had higher settler densities (15.8 ± 3.68 settlers.100 $cm^{-2}$) than glass, ceramic foam, porous concrete, PLA, Aquaroche and FGP. 3DS concrete also had a good success rate (13.2 ± 1.42 settlers.100 $cm^{-2}$), similarly to PVC (13.38 ± 1.56 settlers.100 $cm^{-2}$). Both materials were better than glass, ceramic foam, porous concrete, and PLA. FGP and Aquaroche had similar settler densities (11.65 ± 2.06 settlers.100 $cm^{-2}$, 10.75 ± 2.09 settlers.100 $cm^{-2}$, respectively), which were higher than those of glass and ceramic foam.

After 14 months, settler densities decreased (Fig. 7.2B). The density of surviving settlers averaged 1.07 ± 0.12 settlers.100 $cm^{-2}$ and that of new settlers 0.99 ± 0.09 settlers.100 $cm^{-2}$ There was a significant interaction material x time on total settler density (Table 7.S1, significant interaction Material*Time). Notably, PLA performed better relative to the other materials, such as glass and ceramic foam (Fig. 7.2B).

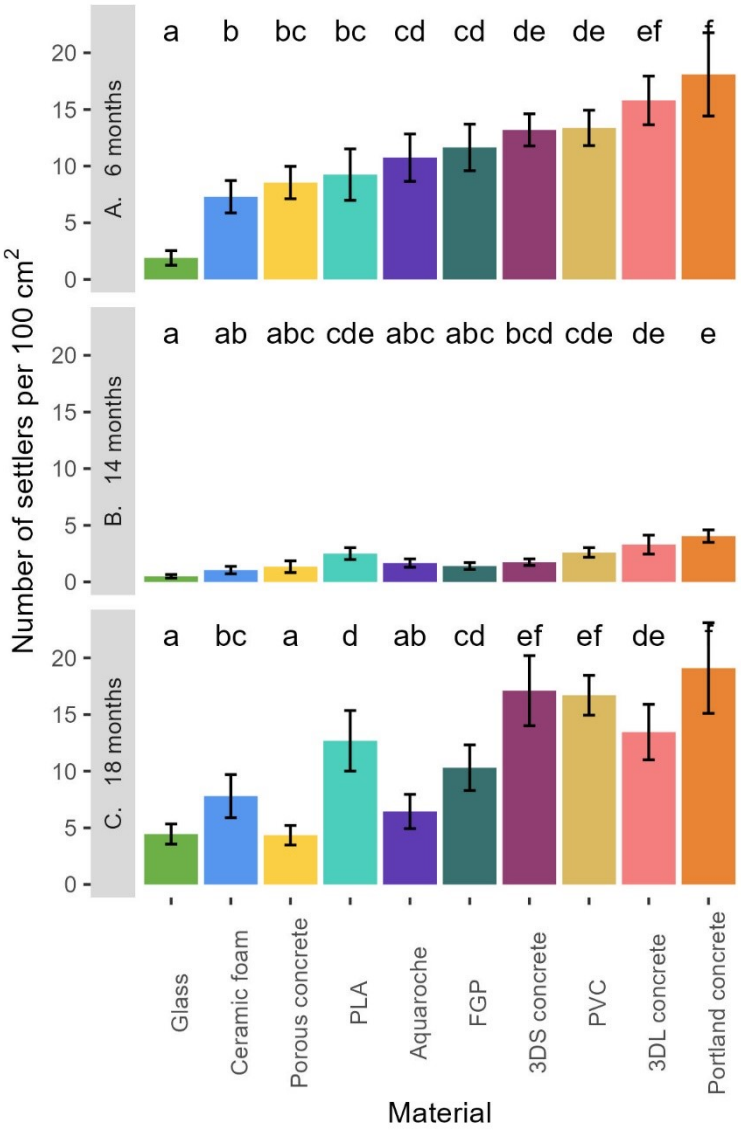



**Figure 7.2.** Total coral settler density (mean ± SEM) on the different materials (sum of new settlers and survivors). Settler density was measured after (A) 6 months, (B) 14 months and (C) 18 months. Letters indicate groups of materials with significantly different settlement based on *emmeans* post hoc tests ($p < 0.05$).

After 18 months, a new wave of coral settlers brought total settler density up to an average of 11.63 ± 0.78 settlers.100 $cm^{-2}$. Of these, survivors represented only 1.04 ± 0.78 settlers.100 $cm^{-2}$. Total settler density was not different than density after six months (KW pvalue = 0.66). After this long conditioning, 3DS concrete and PVC became just as successful as Portland concrete, whereas 3DL concrete became less successful than Portland concrete. PLA also became more successful, more than glass, porous concrete, ceramic foam and Aquaroche. The density on glass increased 2.3-fold after 12 months (4.45 ± 0.89 settlers.100 $cm^{-2}$). On the other hand, the density on porous concrete decreased by 49.1% after 12 months (4.35 ± 0.87 settlers.100 $cm^{-2}$), and on Aquaroche by 46.0% (5.80 ± 1.43 settlers.100 $cm^{-2}$). On the other materials, the density in March 2021 was not statistically different than in March 2022.

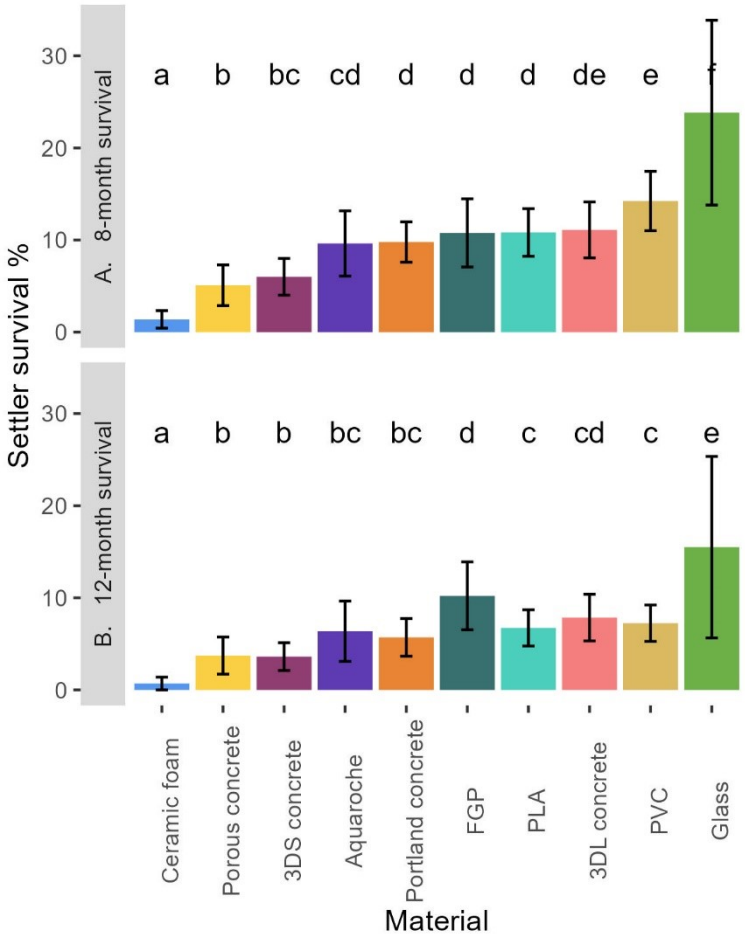



**Figure 7.3.** Survival (mean ± SEM) of the first generation of coral settlers (counted after 6 months) after 8 (A) and 12 more months of immersion in situ (B), on each tested material. Letters indicate groups of materials with significantly different survival based on *emmeans* post hoc tests ($p < 0.05$).

## 3.2 Settler survival

Survival rates were influenced by material and time and their interaction (Table 7.S1B). There was also a significant log-link negative correlation between initial settler density and survival rates (Fig. 7.S3).

Between 6 and 14 months, survival averaged 10.08 ± 1.10%. It was higher on glass tiles (23.83 ± 10.03%) than any other materials (Fig. 7.3A), and it was lower on ceramic foam (1.39 ± 0.95%) than any other material. Survival on PVC was also higher (14.23 ± 3.23%) than for all other materials, except 3DL concrete. 3DL concrete (14.23 ± 3.23%), PLA (14.23 ± 3.23%), FGP (14.23 ± 3.23%) and Portland concrete (14.23 ± 3.23%) all had better survival rates than ceramic foam, porous concrete and 3DS concrete.

Between 6 and 18 months, survival averaged 6.43 ± 0.89%. It varied between material and the interaction material x time was significant (Fig. 7.3B). FGP showed a better 12-month survival

rate than most materials, except 3DL concrete and glass, whereas its 8-month survival was only better than ceramic foam, porous concrete and 3DS.

## 3.3 Settler size and growth

Settler size was influenced by material and time and their interaction (Table 7.S1C). After six months of immersion, glass and PLA tiles displayed the biggest settlers (5.29 ± 1.36 and 4.83 ± 0.87 polyps, respectively), bigger than on ceramic foam, Aquaroche, 3D concrete and porous concrete tiles (Fig. 7.S1A). FGP, PVC and Portland concrete also had bigger settlers than ceramic foam and Aquaroche. After 14 months of immersion, settler size increased 5.2-fold (from 3.01 ± 0.09 polyps at six months to 15.69 ± 1.20 polyps). The materials with the biggest settlers were still glass, FGP, PLA, PVC, and Portland concrete (Fig. 7.S1B). After 18 months, settler size dropped by 55.4% to 7.00 ± 0.66 polyps. PLA, PVC and Portland concrete harbored the biggest settlers, bigger than ceramic foam and 3DS concrete (Fig. 7.S1C).

Settler growth was influenced by material and time, but their interaction was nonsignificant (Table 7.S1D). The 8-month growth of surviving first generation settlers did not vary between materials. However, the 12-month growth was different between materials (Table 7.S1D, Fig. 7.S2). Settlers grew 4.4-fold more on 3DS concrete (by a factor 38.6 ± 17.3) and 2.8-fold more on PVC (24.2 ± 4.1) than on PLA (8.8 ± 1.9) (Fig. 7.S2).

## 3.4 Benthic communities

Benthic community composition changed between materials and times, with a significant interaction between both factors (Table 7.S2, Anova $p < 0.001$). After six months, most materials differed in their benthic community composition, except some such as 3D L and S concretes which had statistically similar communities (Fig. 7.4, Table 7.S3). PVC also had similar communities to PLA and FGP, ceramic foam was similar to Aquaroche, Portland concrete was comparable to FGP, porous concrete to Aquaroche and FGP (Table 7.S3). At 14 and 18 months, materials had less distinguishable communities. Benthic community composition on glass was very different than that of any other materials. Bryozoans, other algae, and tunicates did not vary in abundance on different materials, but all others did. Between 6 and 18 months, there was significantly more turf algae (< 0.001), dead CCA (0.023), foraminiferans (< 0.001), sponges (0.01), wormtubes (< 0.001) and other fauna (< 0.001), and less bare substrate (< 0.001) (Table S4, Fig. 7.S5).


**Figure 7.4.** Benthic cover on the settlement tiles of different materials after 6, 14 and 18 months on the forereef of Mo'orea, French Polynesia.

## 3.5 Influence of benthic communities on coral settlement and survival

There was a significant negative correlation between the cover of bare surface and settler density at both 6 and 18 months (Fig. 7.5). At six months, the abundance of CCA, dead CCA, *Lobophora*, sponges, tunicates, turf algae and worm tubes were positively correlated with settler density. At 18 months, settler density was negatively correlated with the cover of bare substrate and CCA, positively with that of coral settlers, *Lobophora*, turf and other algae. Survival at 8-month was positively correlated only with the cover of coral settlers (after 14 months). Survival at 12 months was also positively correlated with the cover of coral settlers, and negatively with that of bare substrate (Fig. 7.5).

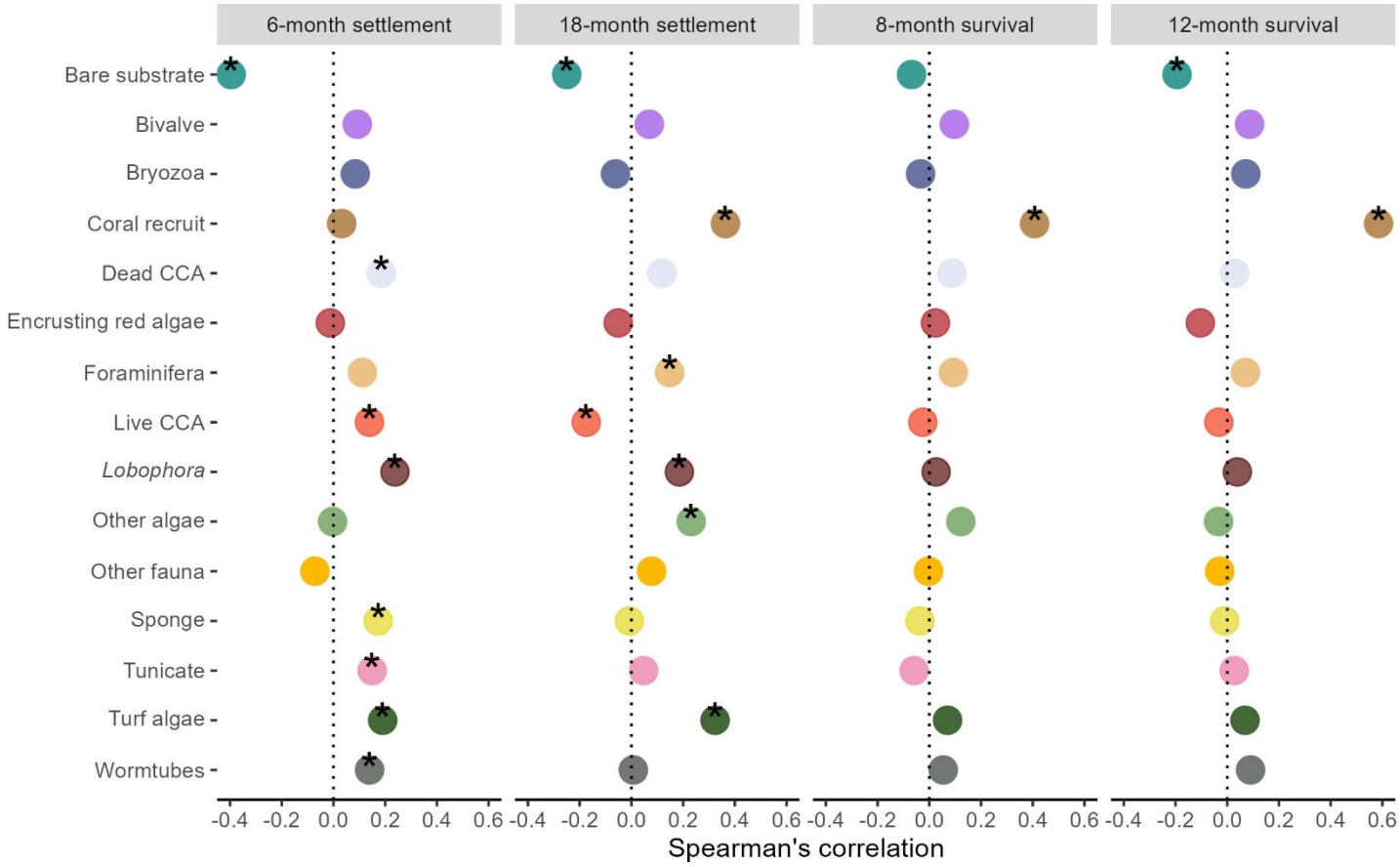



**Figure 7.5.** Spearman's correlations between settler density (after six months) or settler survival (after 8 months) and all benthic categories.

## 4. Discussion

The present experiment aimed to compare the efficiency of different artificial materials as coral settlement substrates and help finetune the properties of artificial materials designed for reef restoration. Settler density and survival significantly varied with material type and immersion time, but materials with the highest settler density did not show the highest settler survival. After 18 months, Portland, 3D-printed concretes and PVC displayed the highest settler density, while glass and fiberglass showed the best survival rates. The covers of *Lobophora*, turf algae and sessile animals were positively correlated with settler density, and the percent of surface left uncolonized on each tile was negatively correlated to settler density and settler survival, indicating that successful materials for corals also attracted an array of benthic communities.

While the relative initial success of materials was comparable to our first experiment (Leonard et al. 2022), settler densities were very different. They were almost 3-fold higher on nearly identical materials in this study (11.2 live settlers.100 $cm^{-2}$ in 2021) compared with the one of Leonard et al. (2022) (3.9 live settlers.100 $cm^{-2}$ in 2020). This increase could be a consequence of the 2019 bleaching event that affected Mo'orea, French Polynesia (Leinbach et al. 2021; Burgess et al. 2021). During this event, 46% of *Pocillopora* and 89% of *Acropora* colonies showed signs of bleaching in Mo'orea (Speare et al. 2022). *A. hyacinthus* colonies that bleached and recovered showed a decreased fecundity the following spawning season (Leinbach et al. 2021). A 2.8-fold decrease in settlement following coral bleaching was also observed on the temporal replicates of a previous experiment in Mo'orea, on horizontal tiles of varying complexities (Chapter 6). An increase of settlers in 2021 was also observed in different sites in Mo'orea (Edmunds et al. 2024). The reproductive output of corals is known to be reduced after bleaching events (Mendes & Woodley 2002; Ward et al. 2002; Hagedorn et al. 2016), which translates into fewer juveniles recruiting than in a normal year (Mallela & Crabbe 2009; Pengsakun et al. 2012; Hughes et al. 2019). It is thus hopeful to consider that settlement rates can increase 3-fold less than two years after an intense coral bleaching event.

After a very high initial mortality following the first sampling, settler density strongly decreased in October before increasing again in February 2022 with the new wave of coral settlers from the summer of 2021-2022. This indicates that settlement on the fore reef between March and October is extremely low (< one new settler.100 $cm^{-2}$). However, total settler density after 18 months was not higher than the one after 6 months. On horizontal exposed tiles, however, subsequent settlement after a total of 21 months immersion was significantly higher than the initial settler density a year earlier (Leonard et al. 2024b). On exposed tiles, the grazing of fish or urchins could potentially regularly free up space by removing competing algae and leaving CCA (Doropoulos et al. 2016; Sampayo et al. 2020), creating more settlement substrate for new settlers compared with the sheltered tiles of this study. Similarly, the peak density after 9-14 months of Arnold and Steneck (2011) occurred on sheltered tiles, just before tiles became fully colonized (Arnold & Steneck 2011). Sheltered tiles might thus show peaks in settlement

followed by declines, as shown by the high densities after 6 and 18 months following the main spawning seasons in this study.

Overall, survival rates were very low, averaging 6.4% between 6 and 18 months. Most coral settler skeletons were overgrown or sloughed out. On tetrapods seeded with coral larvae, survival was comparable, averaging 9.6% after one year in Curaçao (Chamberland et al. 2017) and 6.3% after seven months on the Great Barrier Reef (Randall et al. 2023). Since our vertical tiles were sheltered from grazing, mortality was likely due to overgrowth by competing organisms, such as bryozoans (Chadwick & Morrow 2011), *Lobophora* (Eich et al. 2019), CCA (Harrington et al. 2004; Jorissen et al. 2020), ERA (Pueschel & Saunders 2009; Edmunds et al. 2019) or sponges (Arnold & Steneck 2011). It could also be due to predation by small invertebrates that could reach into the 15 mm gap between tiles, such as corallivorous snails (Hamman 2018) or juvenile urchins (Qiu et al. 2014). We found a nonlinear negative correlation between initial density and survival probability, similarly to previous studies (Doropoulos et al. 2017). This could either be a sign of competition among coral settlers, or reflect competition with other organisms, as tiles with the highest settler density also showed the highest benthic cover.

Benthic community composition can vary in time on artificial materials and take longer than two years to become stable (Svane & Petersen 2001). The variable performance of the materials likely reflects differences in benthic community succession. While most materials had distinct benthic communities after six months, some of these differences disappeared after 18 months. While the abundance of sponges, tunicates and worm tubes were positively correlated with the initial settler density after six months, these correlations were no longer significant after 18 months. It was mostly the cover of older coral recruits that had a positive influence on the 18-month settler density. The presence of older recruits could thus have enhanced the settlement of new settlers, similarly to the clustered recruitment observed in some coral taxa (Doropoulos et al. 2017; Pedersen et al. 2019). The positive correlation between coral cover and survival is understandable because final coral cover was mostly composed of surviving recruits, being significantly larger than new settlers.

Curiously, *Lobophora* showed a positive correlation to settler density, both after 6 and 18 months. This was also the case for 21-month settler density on exposed horizontal tiles (Leonard et al. 2024b). While a negative correlation with settler survival had been observed on exposed tiles (chapter 6), *Lobophora* was not correlated to survival in the present experiment. The influence of *Lobophora* on coral settlement is variable in the literature. This macroalga reduced the ex situ settlement of *Porites* from Florida (Kuffner et al. 2006), Acroporidae and Pocilloporidae from the Great Barrier Reef or Palau (Baird & Morse 2004; Evensen et al. 2019b), but seawater conditioned with *Lobophora* increased *Acropora* settlement by 40% (Birrell et al. 2008). This result could also be due to distinct epiphytic bacterial communities, the provision of cryptic microhabitats under the algal thalli, and/or the production of secondary metabolites acting as deterrents against herbivores or competing organisms (Arnold & Targett 2000; Walters et al. 2003). In the present experiment, coral

settlers were protected from grazing fish, and still settled more on tiles with abundant *Lobophora*. Alternatively, this relationship is not causal and *Lobophora* and coral settlers could prefer the same materials (i.e., FGP, Portland and 3DL concrete).

Turf algae, which were generally thin and sparse, also showed a positive correlation with settler density. This is consistent with the 2020 experiment (Leonard et al. 2022). Thin turf algae are not strong competitors against corals in contrast to thick turf, macroalgae and cyanobacteria (Jompa & McCook 2003; Jorissen et al. 2016), unless they trap large amounts of sediment (Birrell et al. 2005; Speare et al. 2019). Finally, live and dead CCA both had a positive influence on initial settlement. Dead CCA can sometimes induce metamorphosis (Heyward & Negri 1999). However, after 18 months, live CCA was negatively correlated to settler density. This is contrary to the findings of Elmer et al., who observed a positive correlation between settlement and CCA cover after 9 and 15 months (Elmer et al. 2018) and might be due to species-specific differences in the CCA communities between studies.

Our results demonstrate that the properties of artificial materials can significantly influence recruitment, similarly to what other studies observed (Harriott & Fisk 1987; Fitzhardinge & Bailey-Brock 1989). The success of each material after six months in situ had already been previously measured (Leonard et al. 2022), and was comparable in the present study. Materials showed similar success, with Portland concrete and 3DL concrete having the highest settler density, followed by PVC, 3DS concrete and FGP. However, the performance of some material varied over time. For instance, settler densities on glass, PLA, and 3DS concrete relative to other materials increased with time, while those on 3DL, porous concrete, and Aquaroche decreased.

Glass was less successful than any other tested material. In general, glass seems to be an unappealing settlement substrate for coral larvae (Goh 1991; Lei et al. 2021). However, when using unconditioned tiles in a no-choice experiment, settlement on glass tiles is no different than on acrylic, cement, ceramic or PVC tiles (Lee et al. 2009). In another study, glass has been shown to be as adequate as PVC or limestone tiles for CCA growth (Kennedy et al. 2017). The smooth surface of glass probably hinders the formation of a favorable biofilm or the colonization by CCA. This is supported by the fact that 63.3% and 39.1% of the glass surfaces were still bare after 6 and 18 months on the forereef. While glass displayed the lowest settler densities, survival probabilities of coral settlers on glass were the highest of all tested materials, which could be due to reduced competition. The smooth texture of glass might act just as antifouling wax coats which reduce algal growth (Tebben et al. 2014). However, due to its smooth texture, coral settlers settled on glass may run the risk of sliding off as they grow.

Similarly to glass, ceramic foam and Aquaroche were also less colonized than other materials. These materials tended to be very brittle, thus prone to erosion. The erosion observed on these materials likely limits settler survival (Leonard et al. 2022). Likewise, settler survival on *Porites* skeleton slabs was strongly reduced compared to ceramic tiles (Harrington et al. 2004), and sandstone tiles showed signs of erosion and the lowest settlement relative to ceramic and

concrete tiles (Burt et al. 2009). Porous concrete made of small gravels instead of sand was among the worst materials in terms of settler density. When exposed to predation, this material offers the advantage of having many micro-crevices to protect coral settlers from predation (Chapter 6). However, it also attracts many competing organisms.

Overall, the three tested polymers, PVC, PLA with flax fibers and fiberglass with epoxy (FGP), performed well. PVC is a highly durable material that has been shown to favor coral recruitment (Mallela et al. 2017). Epoxy resin can be a durable settlement substrate and attract coral larvae, especially when a certain coarseness is added to the smooth resin (Thomason et al. 2002). Fiberglass structures even show higher coral recruitment than natural coral rubble (Loh et al. 2006). Thin PLA discs can also produce good settlement rates in some coral species (Randall et al. 2024). Interestingly, the success of PLA increased with time. Recruits on PLA, however, grew slower after 12 months than PVC or 3DS. Different 3D-printed polymers can show very similar success. A study testing four filament types (PLA with and without stainless steel shavings, co-polyester based nGen and XT) in 3D printing found no difference in coral settlement preference, 12 week survival or growth (Ruhl & Dixson 2019). After 8 months, PVC also showed good survival probabilities, better than all the materials except glass and 3DL concrete. However, after 12 months, it was FGP that had significantly better survival rates than all materials except glass and 3DL concrete. Smooth materials thus seem to increase survival probabilities compared with more porous or rough materials.

With plastic pollution suffocating the oceans, the ethics of using plastic materials in a restoration project should be carefully considered. The decomposition of most artificial materials releases microparticles, in turn contributing to the transport of pollutants and pathogens in the oceans (Auta et al. 2017). As an example, PVC decomposes through the effect of heat, seawater and UV radiation (Tang et al. 2018). Fiberglass with epoxy resin is very durable in seawater, even after over 7 years of immersion (Mourad et al. 2019). It will, however, eventually turn into microplastics (Lekshmi et al. 2023), which can critically disturb the food chain (Auta et al. 2017). Restoration efforts should thus turn towards biodegradable plastics (Strudwick et al. 2024). PLA is sold as a biodegradable plastic to replace petroleum-based plastics, and its use has skyrocketed in many industries (Taib et al. 2023). Although PLA is made from renewable resources, it is not biodegradable in conventional composting, with natural microbes at ambient temperatures (Tokiwa & Calabia 2006). Pure PLA does not degrade in seawater after 180 days (Huang et al. 2020) and showed no obvious degradation after 18 months in the present study even when mixed to flax fibers. While it is highly durable, it may also have negative impacts on the marine food chain.

Similarly to our previous experiments (chapter 5 and 6), materials made of concrete showed good settler density, but performed less in terms of settler survival. The only concrete that showed good survival rates was 3DL concrete. 3DL concrete might have a more favorable composition or shape thanks to its deeper ridges. It also displayed fewer competitors. Another factor to consider before planning the construction of large artificial reefs is the massive carbon emissions of the concrete industry (Worrell et al. 2001). 3D printing concrete has a

lower environmental impact than conventional concrete construction (Mohammad et al. 2020). Ceramics have been suggested as a more ecological alternative. They can also be 3D printed and show good settlement rates (Berman et al. 2023a). Clay has also been suggested as a low-cost alternative to ceramic materials (Hoog Antink et al. 2018). While we did not test compact ceramic or clay tiles, they might have shown comparable or better results than concrete tiles (Burt et al. 2009).

In conclusion, our results highlight trade-offs displayed by artificial materials in terms of coral density, survival, growth and cover of fouling organisms. 3DL concrete and PLA could be promising materials applied in future restoration efforts because of their durability, high settler density and survival rate, and their potentially lower environmental cost than PVC, fiberglass, and Portland concrete. If artificial reefs can significantly improve coral cover and recruitment rates, they do not address the major underlying threats to coral reefs. As a priority, climate change needs to be urgently addressed if coral reefs are expected to maintain their invaluable ecosystem services in the future.

## Acknowledgments

This study was made possible thanks to the staff of the CRIOBE research station, and was supported partially by the AFR grant from the FNR Luxembourg, as well as partially by SEABOOST through the “Reef” Project and the Agence Nationale de la Recherche (No. ANR-18-CE02-0009-01) and TotalEnergies R&D. We also recognize and give thanks for the land and water resources of Mo’orea, French Polynesia, and to the traditional owners of the land.

## 5. Supplementary material

**Table 7.S1.** Results of Anova on model (glmer models, with unit as the random factor)

| Response variable | Distribution | Explanatory | Chisq | Df | Pr(>Chisq) |
|---|---|---|---|---|---|
| A. Total settler density | Neg binomial | Material | 192.50 | 9 | < 0.001 |
| | | Time (6, 14 or 18 mo) | 464.32 | 2 | < 0.001 |
| | | Material * Time | 30.93 | 18 | 0.029 |
| B. Survival chance | Poisson | Material | 597.95 | 9 | < 0.001 |
| | | Time (8 or 12 mo) | 155.99 | 1 | < 0.001 |
| | | Initial settler density | 27.46 | 1 | < 0.001 |
| | | Material * Time | 28.51 | 9 | < 0.001 |
| C. Settler size (square root transformed) | Neg binomial | Material | 93.79 | 9 | < 0.001 |
| | | Time | 323.64 | 2 | < 0.001 |
| | | Material * Time | 38.89 | 18 | 0.003 |
| D. Settler growth | Neg binomial | Material | 39.37 | 9 | < 0.001 |
| | | Time | 79.33 | 1 | < 0.001 |
| | | Material*Time | 10.50 | 9 | 0.311 |

**Table 7.S2.** Results of Anova test on mvabund manyglm models, with a negative binomial distribution.

| Response variable | Explanatory | Dev | Df | Pr(>Dev) |
|---|---|---|---|---|
| **A. Benthic diversity** | **Material** | **1265.5** | **9** | **< 0.001** |
| | **Time** | **321.6** | **2** | **< 0.001** |
| | **Material*Time** | **482.6** | **18** | **< 0.001** |

**Table 7.S3.** Significant differences (stepdown adjusted p-value from the pairwise anova on mvabunds'manyglm) between benthic communities on different materials after 6, 14 and 18 months in situ. Comparisons in bold are significantly different ($p < 0.05$).

| Time | 6 months | | 14 months | | 18 months | |
|---|---|---|---|---|---|---|
| **Comparison** | **Dev** | **Pr(>Dev)** | **Dev** | **Pr(>Dev)** | **Dev** | **Pr(>Dev)** |
| 3DL concrete vs. 3DS concrete | 27.85 | 0.148 | 21.27 | 0.672 | 22.57 | 0.296 |
| **3DL concrete vs. Aquaroche** | **39.76** | **0.024** | **52.71** | **0.004** | **46.96** | **0.011** |
| **3DL concrete vs. Ceramic foam** | **84.38** | **< 0.001** | **72.51** | **< 0.001** | 28.26 | 0.156 |
| **3DL concrete vs. FGP** | **46.28** | **0.007** | 18.47 | 0.707 | 16.37 | 0.336 |
| **3DL concrete vs. Glass** | **239.20** | **< 0.001** | **124.31** | **< 0.001** | **118.23** | **< 0.001** |
| **3DL concrete vs. PLA** | **81.28** | **< 0.001** | 25.22 | 0.541 | 37.74 | 0.063 |
| **3DL concrete vs. Porous concrete** | **79.34** | **< 0.001** | 38.21 | 0.068 | **50.31** | **0.008** |
| **3DL concrete vs. Portland concrete** | **49.03** | **0.003** | 16.38 | 0.707 | 24.84 | 0.244 |
| **3DL concrete vs. PVC** | **71.08** | **< 0.001** | 28.84 | 0.326 | 33.78 | 0.091 |
| **3DS concrete vs. Aquaroche** | **45.78** | **0.008** | 21.68 | 0.672 | 18.10 | 0.336 |
| **3DS concrete vs. Ceramic foam** | **60.07** | **< 0.001** | **41.84** | **0.028** | 35.75 | 0.079 |
| **3DS concrete vs. FGP** | **62.70** | **< 0.001** | 9.52 | 0.964 | 32.82 | 0.099 |
| **3DS concrete vs. Glass** | **233.46** | **< 0.001** | **98.77** | **< 0.001** | **100.22** | **< 0.001** |
| **3DS concrete vs. PLA** | **104.96** | **< 0.001** | 22.59 | 0.647 | 30.18 | 0.129 |
| **3DS concrete vs. Porous concrete** | **65.40** | **< 0.001** | 30.55 | 0.243 | 34.18 | 0.091 |
| **3DS concrete vs. Portland concrete** | **50.72** | **0.003** | 30.90 | 0.243 | **46.32** | **0.012** |
| **3DS concrete vs. PVC** | **106.60** | **< 0.001** | 22.73 | 0.647 | **49.00** | **0.01** |
| Aquaroche vs. Ceramic foam | 24.60 | 0.205 | 21.23 | 0.672 | 32.61 | 0.099 |
| Aquaroche vs. FGP | 22.92 | 0.205 | 33.71 | 0.133 | **51.40** | **0.008** |
| **Aquaroche vs. Glass** | **181.42** | **< 0.001** | **70.98** | **< 0.001** | **89.59** | **< 0.001** |
| **Aquaroche vs. PLA** | **68.81** | **< 0.001** | **49.31** | **0.006** | **47.38** | **0.011** |
| Aquaroche vs. Porous concrete | 34.86 | 0.053 | 36.03 | 0.085 | 32.00 | 0.105 |
| **Aquaroche vs. Portland concrete** | **54.98** | **< 0.001** | **76.48** | **< 0.001** | **78.13** | **< 0.001** |
| **Aquaroche vs. PVC** | **51.58** | **0.002** | **61.97** | **0.002** | **86.10** | **< 0.001** |
| **Ceramic foam vs. FGP** | **43.47** | **0.01** | **47.70** | **0.008** | **55.82** | **0.004** |
| **Ceramic foam vs. Glass** | **171.81** | **< 0.001** | **55.84** | **0.003** | **69.27** | **< 0.001** |
| **Ceramic foam vs. PLA** | **86.12** | **< 0.001** | **51.29** | **0.006** | 29.27 | 0.141 |
| **Ceramic foam vs. Porous concrete** | **45.72** | **0.008** | **59.60** | **0.003** | **50.69** | **0.008** |
| **Ceramic foam vs. Portland concrete** | **71.39** | **< 0.001** | **81.10** | **< 0.001** | **85.19** | **< 0.001** |
| **Ceramic foam vs. PVC** | **74.78** | **< 0.001** | **63.54** | **0.002** | **79.03** | **< 0.001** |
| **FGP vs. Glass** | **142.85** | **< 0.001** | **112.38** | **< 0.001** | **130.92** | **< 0.001** |
| FGP vs. PLA | 24.97 | 0.205 | 10.25 | 0.964 | **44.61** | **0.021** |
| FGP vs. Porous concrete | 34.65 | 0.053 | 37.18 | 0.077 | **60.46** | **0.002** |
| FGP vs. Portland concrete | 30.21 | 0.112 | 20.49 | 0.672 | 20.81 | 0.309 |
| FGP vs. PVC | 18.01 | 0.205 | 17.79 | 0.707 | 36.11 | 0.074 |
| **Glass vs. PLA** | **181.58** | **< 0.001** | **102.83** | **< 0.001** | **70.61** | **< 0.001** |
| **Glass vs. Porous concrete** | **247.96** | **< 0.001** | **106.49** | **< 0.001** | **123.06** | **< 0.001** |
| **Glass vs. Portland concrete** | **224.68** | **< 0.001** | **146.34** | **< 0.001** | **175.12** | **< 0.001** |
| **Glass vs. PVC** | **232.00** | **< 0.001** | **153.05** | **< 0.001** | **165.68** | **< 0.001** |
| **PLA vs. Porous concrete** | **54.96** | **< 0.001** | 37.88 | 0.07 | **65.46** | **< 0.001** |
| **PLA vs. Portland concrete** | **52.33** | **0.002** | 24.92 | 0.551 | **58.46** | **0.002** |
| PLA vs. PVC | 28.48 | 0.14 | 8.75 | 0.964 | 26.93 | 0.177 |
| **Porous concrete vs. Portland concrete** | **80.21** | **< 0.001** | **66.31** | **0.002** | **82.39** | **< 0.001** |
| **Porous concrete vs. PVC** | **70.35** | **< 0.001** | **60.84** | **0.003** | **90.97** | **< 0.001** |
| **Portland concrete vs. PVC** | **43.01** | **0.011** | 24.30 | 0.557 | 33.87 | 0.091 |

**Table 7.S4.** Results of manyglm comparing abundances of different benthic communities between 6 and 18 months

| Species | Dev | p-value |
|---|---|---|
| Bryozoans | 1.583 | 0.542 |
| **Bare substrate** | **29.999** | **0.001** |
| CCA | 4.110 | 0.202 |
| **Dead CCA** | **8.237** | **0.023** |
| Encrusting red algae | 0.821 | 0.678 |
| **Forams** | **62.271** | **0.001** |
| Lobophora | 0.651 | 0.678 |
| Other algae | 0.953 | 0.678 |
| **Other fauna** | **26.200** | **0.001** |
| **Sponge** | **10.212** | **0.01** |
| **Turf** | **22.112** | **0.001** |
| Tunicates | 3.359 | 0.247 |
| **Worm tubes** | **26.066** | **0.001** |

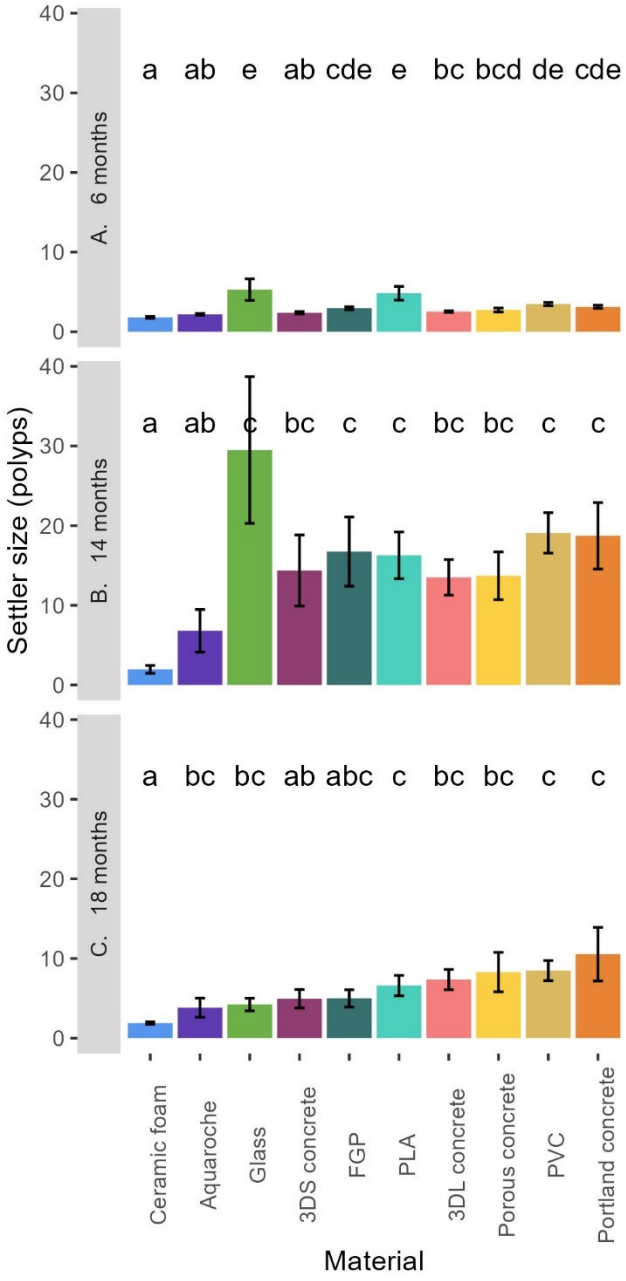



**Figure 7.S1.** Size (number of polyps, mean ± SEM) of all coral settlers on sheltered settlement tiles of different materials, after (A) 6 months of immersion on the forereef of Mo'orea, French Polynesia, (B) 14 months of immersion, (C) 18 months of immersion. Letters indicate groups of materials with significantly different survival based on pairwise Wilcoxon post hoc tests ($p < 0.05$).

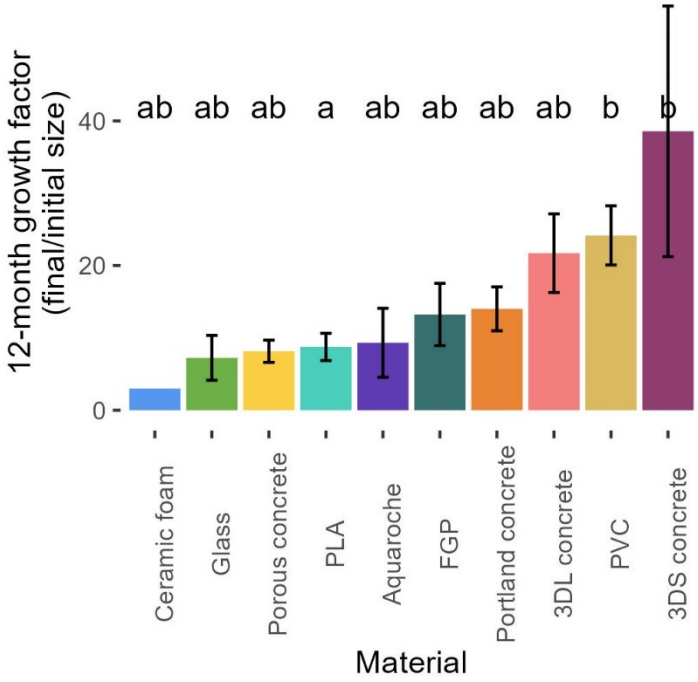



**Figure 7.S2.** Twelve-month growth factor (mean ± SEM) of coral settlers (final / initial number of polyps) on settlement tiles of different materials on the forereef of Mo'orea, French Polynesia. Letters indicate groups of materials with significantly different survival based on emmeans post hoc tests ($p < 0.05$).

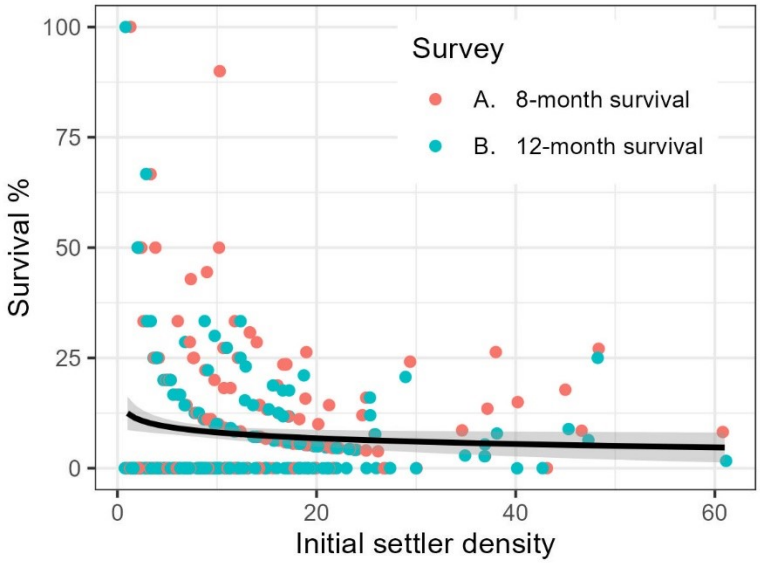



**Figure 7.S3.** Negative log link between settler survival and initial settler density

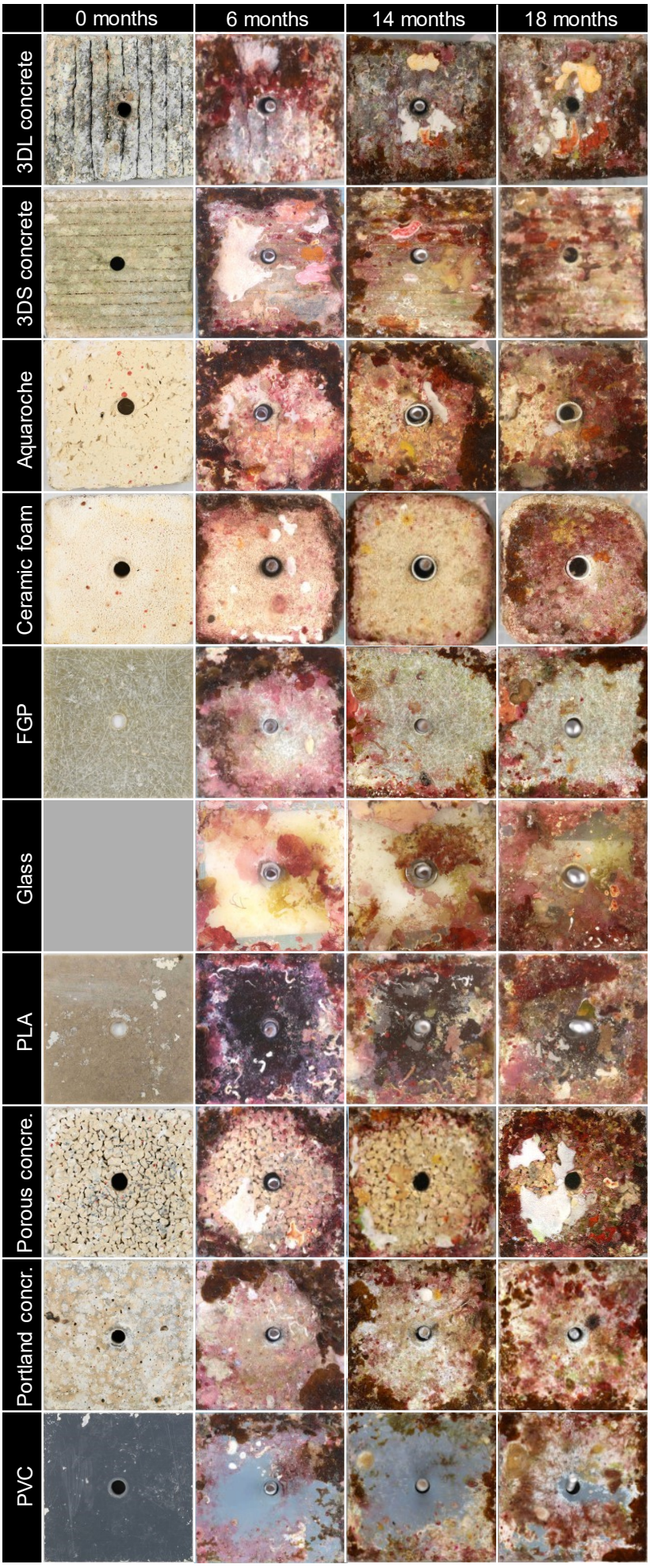



**Figure 7.S4.** Representative tiles collected in the study, showing benthic communities on the different materials before, 6 months, 14 months and 18 months after immersion in the field. Benthic communities varied significantly among materials and among sampling times.

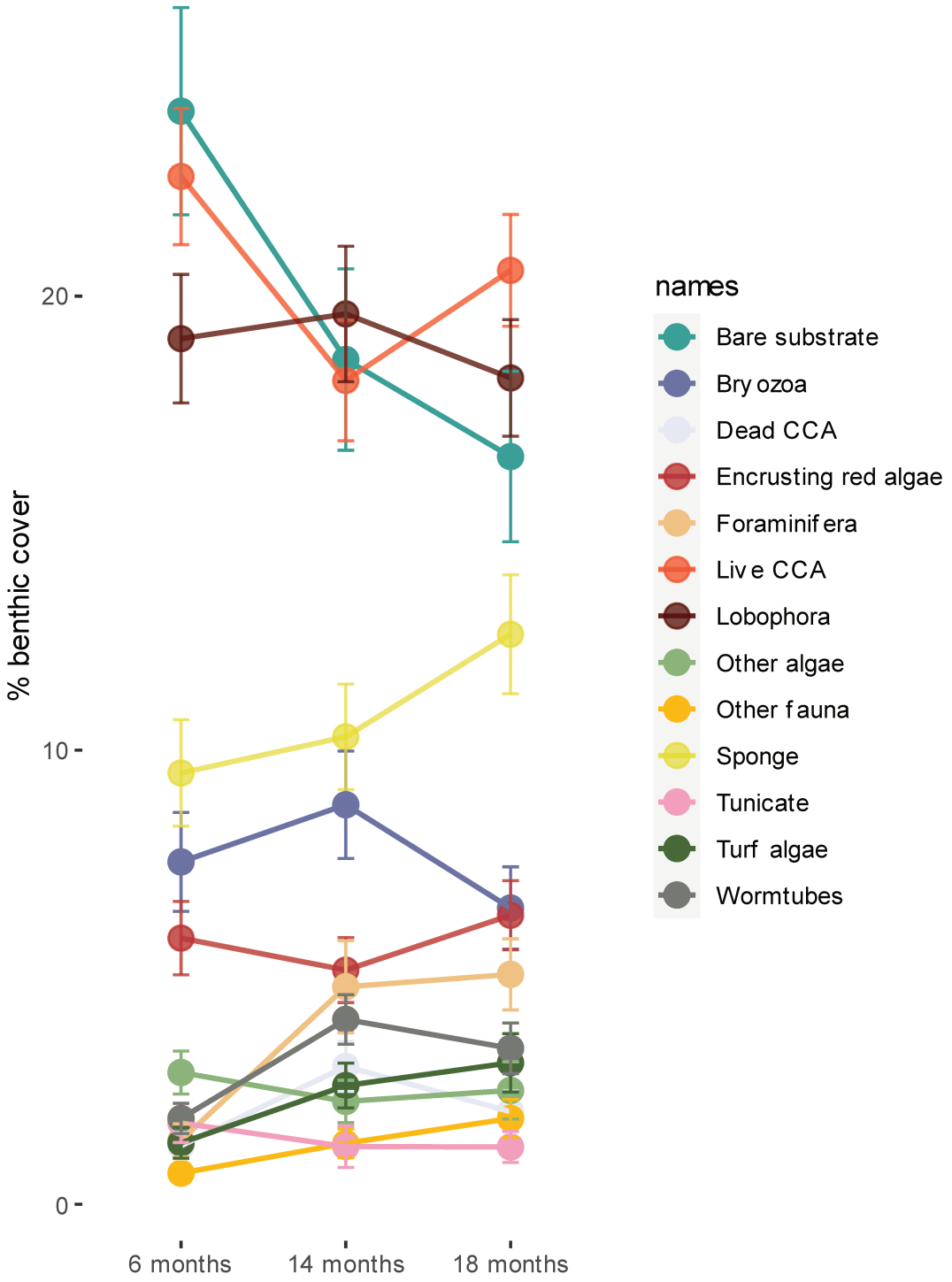



**Figure 7.S5.** Evolution of the benthic cover of vertical settlement tiles on the forereef of Mo'orea, French Polynesia, after 6, 14 and 18 months. Error bars represent 95% confidence interval.

# Chapter 8

## General discussion & perspectives

Coral reefs are nowadays facing unprecedented threats with increasingly frequent massive bleaching events (Reimer et al. 2024). Nature based solutions that will inspire innovative marine restoration are urgently needed to face the current coral crisis. Coral restoration techniques now show great progress since the first attempts at coral gardening 30 years ago (Bowden-Kerby 1997; Smith & Hughes 1999). Most restoration projects still rely either on asexually propagated fragments following transplantation to degraded sites, or substrate enrichments through artificial structures, while only a few projects experimented with using sexual reproduction (Boström-Einarsson et al. 2020a). Large artificial substrate enrichment and transplantation efforts have led to whole reefs being restored to the same coral cover and carbonate production than undamaged sites in just four years (Lange et al. 2024), but the scalability of traditional restoration to whole reef ecosystems remains to be demonstrated.

Building on available knowledge, the overarching goal of my thesis was to study and evaluate factors most influencing the success of different restoration practices trialed in Mo'orea, French Polynesia. The goal was to provide information for targeting restoration efforts towards species or certain genotypes that are most likely to thrive, identify sites for the restoration and the outcome of various stages of restoration in the context of French Polynesia. This region of the world is still largely underrepresented in restoration research (Boström-Einarsson et al. 2020a, 2020b). We aimed to identify the major limitations of each practice in order to improve their outcome in the future and ultimately enhance the resilience of coral reefs in French Polynesia. This PhD thesis presents French Polynesia as an ideal location for the study of coral restoration. Despite frequent disturbances, French Polynesian reefs present a high resilience capacity (Adjeroud et al. 2018). If Mo'orea's coral populations were to drastically plummet, the recolonization could be facilitated by the close proximity of the neighbor island Tahiti (Bramanti & Edmunds 2016). On more remote and isolated islands, however, the shipping and transplantation of missing species might be the only way to restore diversity after a disturbance. Nonetheless, the recent COTS invasion observed in Mo'orea followed by the 2024 global bleaching event (Reimer et al. 2024) might have harmed the Polynesian reefs for the foreseeable future. While the full diversity of these reefs is still accessible, increased restoration efforts should aim to maintain a healthy reserve population of each species in sheltered nurseries. Also, the most endangered species should be identified and multiplied to prevent their extinction.

Branching corals and *Acropora* in particular are usually the first casualties of massive mortality events, be it from COTS predation (De'ath & Moran 1998; Pratchett 2001) or heat waves (Loya et al. 2001; Speare et al. 2022). They are, however, essential for building complex habitats for numerous reef organisms and are indispensable for reef growth and coastal protection (Vytopil & Willis 2001; Kerry & Bellwood 2012; Young et al. 2012). Because of their sensitivity, their abundance is constantly declining in many sites around Mo'orea (Trapon et al. 2011). The restoration of *Acropora*, which is the underlying goal of the first three chapters of this PhD, could thus become necessary to recover the initial biodiversity and productivity of some degraded sites on Polynesian reefs, and to ensure the durable protection through barrier reefs

of coastal communities, that are so abundant in French Polynesia. The corals grown in the nursery used in the first three chapters of this thesis are corals that have previously demonstrated bleaching resistance. In fact, while almost 50% of the nursery corals died during the 2019 bleaching event in Mo'orea, the ones that survived proved to be more tolerant and thrived after the bleaching event. The selection of coral genotypes for their heat resistance could be a promising way to increase the resilience of restored areas in the future (Quigley et al. 2020; Howells et al. 2021). This is why these specific nursery-grown corals were used in the first three experiment instead of random fragments. We also noticed that most restoration studies still focus on only three species, *Acropora cervicornis*, *Pocillopora damicornis* and *Stylophora pistillata*. Our experiments will thus improve general knowledge on the nursery rearing and breeding of less represented species in restoration studies, which is crucial to allow the application of restoration practices to Polynesian ecosystems.

## Key messages of this PhD

To briefly summarize the results of previous experiments, chapter 2 found that forereef nurseries could be a good alternative to lagoon nurseries in case the conditions in the lagoon environment were to degrade. Chapter 3 found that transplantation of corals after a two-year nursery phase could result in similar survival, growth, and reproduction rates as in the nursery, after an initial acclimation phase of around one year where growth was strongly impaired through predation and transplantation stress. Chapter 4 found that overall nursery populations maintained a normal spawning pattern and reproductive output relative to wild populations. We also found that fertilization rates can be significantly improved by crossing known compatible genotypes. Chapter 6 found that increased surface complexity improved settlement and recruit survival, but the smoothness of the artificial material strongly influenced the benthic colonization which can compromise recruit survival. Finally, chapter 5 and 7 found several artificial materials that can be good candidates for AR design through their durability, high attractiveness for coral larvae and low colonization of competing organisms.

## Integrative approach of restoration results

The present PhD introduced a large quantity of results concerning restoration techniques that should not necessarily be stand-alone practices. All techniques described in this PhD could in fact be combined with one another to improve their outcome (Fig. 8.1). Ouplanting coral species to an area where they have disappeared could be a way to ensure local larval supply when they become mature, which is usually less than two years after transplantation (Okubo et al. 2009; Young et al. 2012), as shown in chapter 3. After installation, restoration projects should become self-sufficient and not require maintenance. Once an initial coral population is established, the total coral recruitment should constantly increase, through local recruitment or attraction of larvae by adult colonies (Muko & Iwasa 2011a), until the system reaches a state of equilibrium, similar to natural unharmed reefs.

A lack of long-term monitoring in most restoration projects leads to almost no reports of spawning behavior and reproductive output of restored coral, which is, however, an important indicator of the restoration success and reef replenishment (Hein et al. 2017). The maturity onset was observed in most corals reaching a species-specific maturity size in chapters 2 and 3, and the reproductive output of nursery corals proved to be similar to that of wild corals in chapter 4. This shows that nurseries and outplanted corals can become efficient reservoirs of larvae to improve the genetic diversity of impoverished sites.

Larval production of outplanted colonies could in turn improve the colonization of ARs (artificial reefs) in areas where recruitment is otherwise low, or fragments could be directly used to increase coral cover on the artificial structure and attract more larvae (Fig. 8.1). Other studies for instance showed high survival rates of coral fragments planted directly onto artificial substrates (Fadli et al. 2012; Williams et al. 2019; Knoester et al. 2023), likely because these present less competitive organisms than natural substrates. We found in chapter 7 that the abundance of one-year-old recruits was enough to be positively correlated with new waves of recruits, potentially attracting larvae through conspecific chemical cues (Doropoulos et al. 2017; Pedersen et al. 2019). This can produce a domino effect in attracting more and more larvae until ARs reach a state of equilibrium.

ARs can present many benefits, increasing the complexity, recruitment rates and biodiversity of a site (Yanovski & Abelson 2019). They can be raised from the seafloor in order to resist COTS invasions or be populated by artificially selected heat tolerant corals to resist bleaching events. In addition, they can become new diving sites and be used to attract diving pressures away from more sensitive areas that need to recover (Oh et al. 2008; Tynyakov et al. 2017; Firth et al. 2023), for instance those containing fragile and freshly transplanted corals. The benefit of removing human pressures in protected areas for reef recovery has been shown in many areas (McCook et al. 2010).

Increased research on the optimal artificial materials for coral settlement and survival will undoubtedly improve the outcomes of AR design, but also future restoration practices using sexual reproduction. Chapters 5 to 7 found that sheltered surfaces harbored particularly high densities of recruits, with hard rougher materials such as 3D or Portland concrete being favored relative to the very smooth surface of glass or the very porous ceramic foam or porous concrete. However, survival was enhanced on upper sides with crevices and on smooth materials, that harbored less competition than porous ones (chapter 6). This could help to further improve the survival of seeded larvae, as we observed extremely high post-settlement mortality rates in chapter 4 because natural substrates often lack complexity that can protect larvae from predation.

In return, assisted larval seeding could also be an efficient way to significantly increase coral settlement on an AR, especially in areas where natural recruitment is extremely low, like we measured in the lagoon of Mo'orea in chapter 4. Seeding degraded bommies with larvae can produce similar recruit density after just ten days in the lagoon (chapter 4), as after six months

on the fore reef (chapter 7), where natural recruitment rates were substantially higher than in the lagoon thus no artificial seeding would be needed. Similarly, larval seeding on concrete blocks can produce good recruitment rates even in an anthropized port, where no recruits were observed on control plots (Omori et al. 2003).

As they are easily replicable, transplantation and AR deployment are the most used restoration practices, and there is a lack of accessibility concerning restoration using sexual reproduction. We aimed to demonstrate that a simple protocol at a small scale can be easily replicated by using common and accessible tools in chapter 4. We showed that small hand-sewn tents, maintained upright by chain and bicycle tubes for only two days and filled with larvae from plastic bottles through a hose and funnel, can be enough to significantly improve settlement rates in degraded lagoon sites. This method could be easily employed by restoration NGOs that currently only use transplanting of adult corals in order to improve the long-term coral cover. The seeding of larvae or of sexually obtained juveniles can in fact be more cost-effective than transplanting adult corals (Baria-Rodriguez et al. 2019; Doropoulos et al. 2019a). Furthermore, it can be used to seed specifically selected larvae, for instance those that inherit heat-resistance from their parents.

With the presented experiments, nursery rearing proved to yield corals with the same reproductive output as undisturbed wild colonies. This is significant, as it will allow to combine restoration techniques using both asexual and sexual reproduction. Coral nurseries can be both sources of asexual fragments for outplanting, and sources of gametes during spawning events. This could alleviate pressures on fragile wild populations by removing the need to sample them to produce larvae ex situ. Furthermore, the fitness of nursery and ouplanted colonies could be enhanced by artificially selecting or cross-breeding certain genotypes through more research in assisted evolution. For example, we found that through gamete compatibility, certain parents produced significantly more viable embryos even at high temperatures in chapter 4, which could potentially turn into juveniles with an enhanced thermal tolerance. By integrating all the gathered results, this PhD thesis represents an encouragement to try and combine different restoration techniques in the future instead of only focusing on one aspect (Fig. 8.1).

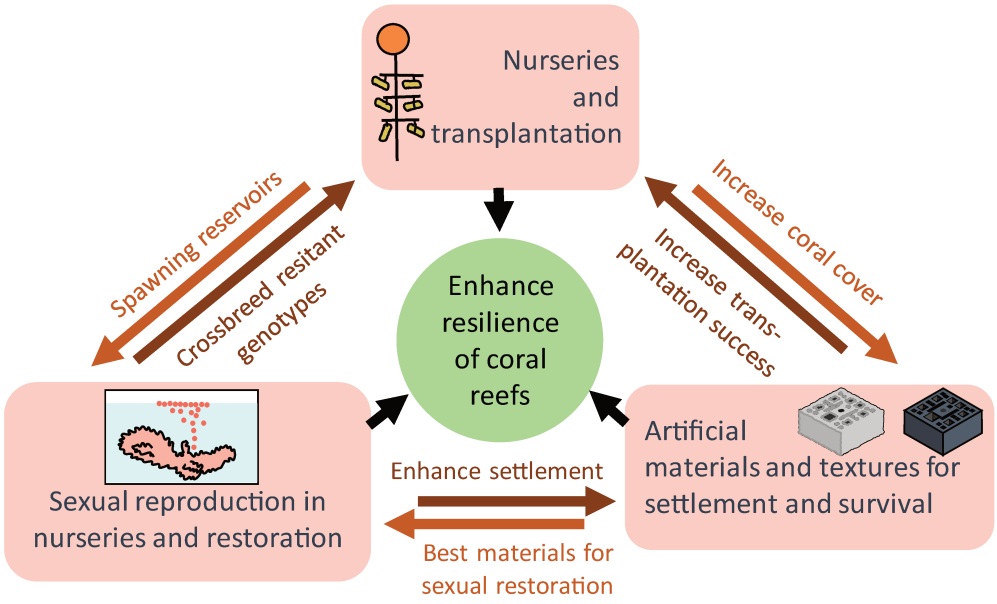



**Figure 8.1.** Summary of potential links and synergies between restoration techniques investigated in the present PhD thesis.

## Recommendations for restoration

Coral reef restoration is among the most expensive ecosystem restoration, up to USD 2 million/ha (Bayraktarov et al. 2015), but costs vary greatly depending on the method used. The high financial cost of restoration can be a significant hurdle, and it is the biggest concern of restoration practitioners, who regret a lack of funding (Young et al. 2012). A careful planning and optimization of output could improve the cost-effectiveness of restored corals relative to what was estimated in this PhD.

For example, in coral nurseries, material failure during strong swells on the fore reef could be prevented by using appropriately durable tools. Nurseries can also be protected by moving them to deeper sites during storms (Johnson et al. 2011). If conditions in lagoons are unfavorable, we showed in chapter 2 that fore reef nurseries as deep as 14 m can be as good as lagoon nurseries for the survival and growth of corals, and can benefit from better water quality and less algal competition. This result could, however, be specific to *Acropora*, as *Pocillopora* can display a preference for lagoon environments (Dehnert et al. 2022).

Considering the strong size-dependent growth and maturity onset of nursery corals, we concluded in chapter 2 that small fragments should be favored to boost growth rates, and fast-growing species such as *A. hyacinthus* and *A. nasuta* could be used for their rapid maturity onset. These species would be good candidates to optimize return-on-effort in coral nurseries, as they showed both high growth rates and high probabilities to be mature in chapter 2. They also proved to have the same reproductive output as wild colonies in chapter 4. Fast growth can be beneficial for nursery yield, for habitat construction and for reef accretion for coastal protection once outplanted, rapidly improving coral cover and reproduction output thus the success of the restoration (Muko & Iwasa 2011a).

Other species we experimented on, such as *A. retusa* and *A. globiceps,* would be less ideal for restoration purposes because they showed a slower growth in chapter 2, and because they either had low maturity rates in chapter 2 or low fertilization rates relative to wild populations in chapter 4. *A. striata* may grow fast but was almost never observed with eggs, indicating that it might need to reach a much larger size before maturity, which is not compatible with restoration aiming to combine asexual and sexual reproduction. *A. cytherea* also showed the need to reach a large size before reaching maturity in chapter 3, and should ideally be grown out in nurseries until they reach about 200 $cm^2$ in order to enhance their survival probability and maturity onset once outplanted. Considering the high mortality observed in chapter 3, the transplanting of more colonies (more than 24 per site) would yield more survivors after two years thus improve the cost effectiveness of restoration efforts, as cost effectiveness is directly linked to the number of outplants and their survival rate (Edwards et al. 2010).

There is a risk that repopulating a site with only *Acropora* could lead to widespread mortality following a COTS invasion or intense bleaching (Muko & Iwasa 2011a). In consequence, the transplantation of more than one species or genus should be considered in order to better improve the diversity of a site. Different species transplanted together could improve growth rates or survival rates through a better resistance to wave damage or COTS predation (Cabaitan et al. 2015; Clements & Hay 2021). However, in some cases, *Acropora* and *Porites* can hinder the recovery of endangered species (Muko & Iwasa 2011a). More transplantation focused on short-dispersal brooding species or slow-growing species could also facilitate their recovery in certain sites (Muko & Iwasa 2011a, 2011b). Identifying ecosystem services associated with trait diversity and depleted species in each location should allow to pinpoint the species to use in restoration (Madin et al. 2023).

Preliminary studies on lagoon recruitment rates could inform restoration practitioners on the sites most lacking in coral replenishment in the future, thus identifying the sites that would benefit most from transplantation (Edwards & Clark 1999). For instance, we measured *Acropora* recruitment in the lagoon at Mahana on seeded and control bommies (dead massive corals) in chapter 4. After ten days, 12, 16 or 20 months, not a single *Acropora* recruit was observed on any of the six control bommies, which measured between 40-120 cm in diameter. This confirms the extremely low natural recruitment rate in this site in the lagoon, similarly to previous studies in the North shore of Mo'orea (Edmunds et al. 2010; Bulleri et al. 2018). This

indicates that transplantation might become necessary in order to help lagoon populations recover and increase the live coral cover. Despite low recruitment and low coral cover, we demonstrated in chapter 3 that the absence of certain keystone species in a site does not exclude their survival when transplanted to those sites. Thus, even sites considered poorer than reference sites can successfully be restored through transplantation. However, as sedimentation and algal competition can reduce coral survivorship (Dizon & Yap 2006), sources of pollution should be managed in parallel to restoration efforts.

Nonetheless, an enhancement of coral larvae could be more efficient at increasing the long-term coral cover than the costly transplantation of adults. Seeding thousands of coral larvae obtained from just a few colonies directly onto the reef (dela Cruz & Harrison 2017) could be an effective way to bypass the need for a long nursery phase and a transplantation phase, thus reducing the labor time by a factor ten (chapter 4). The survival of recruits in seeding projects could be further improved if more shelter were available for the protection of young settlers. Indeed, crevices have been shown to durably improve recruit survival on exposed surfaces in chapter 6, similarly to previous studies (Nozawa 2008; Randall et al. 2021). Substrate for larval enhancement could have been improved by a better choice of bommies to restore, or the adding of crevices by drilling holes into the bommy.

The results of our recruitment experiments allowed us to make informed recommendations concerning AR design. First, artificial reefs should display many shelters to protect recruits from grazing fish, but only on upper or vertical sides. In fact, all sides facing down should be as smooth as possible, either in smooth concrete, hard ceramic or a plastic polymer. We observed that recruits settled more on lower flat surfaces than on lower textured ones, potentially because textured ones harbor more varied competitive communities than flat surfaces. Second, the shelters on upper and vertical sides should not be made like the porous concrete we tested, that is a kind of conglomerate of small pebbles (< 10 mm) and cement. Indeed, porous concrete showed significantly lower survival rates than PVC with printed holes, likely because it also attracted many competitive taxa like bryozoans, sponges, ERA and *Lobophora*, unlike PVC. These taxa are known to rapidly overgrow coral recruits (Chaves-Fonnegra & Zea 2011; Elmer et al. 2016; Evensen et al. 2019b; Edmunds et al. 2019), which could explain their low survival on porous concrete. On the contrary, printed holes in smooth PVC significantly increased survival of recruit on exposed sides. Third, the crevices added to upper and vertical surfaced should be over 5 mm and under 20 mm wide and deep, as this can produce the best shelter for corals, allowing them to grow to a certain size-escape threshold (Raymundo & Maypa 2004; Doropoulos et al. 2012) before outgrowing the shelter and being exposed to predation.

Thus, materials with similar smoothness to PVC could be used instead of concrete with holes, to avoid unwanted competitors. We found very little differences in success between PVC, fiberglass and PLA, which all have similar smoothness and durability, in recruit density or survival. PLA reinforced with flax fibers has the advantage of being made from biological resources, and could be a more ecological alternative to PVC. Both PVC and concrete are not

advisable for restoration if the carbon footprint of the restoration material is taken into account (Worrell et al. 2001; Caldas et al. 2021). In fact, using high carbon footprint materials or transport methods seems counter-productive if the aim is the protection of corals, as the major threat to their survival are our increasing GHG emissions. Nowadays, new recipes for concrete, such as those tested in chapter 7, could produce significantly less carbon emissions than traditional concrete (Mohammad et al. 2020).

In addition to its efficiency, the material should also be tested for its durability in seawater. We found that bioinspired Aquaroche, which is designed to mimic living rocks in the aquarium trade, and ceramic foam mimicking coral skeletons, to be very friable and prone to erosion in seawater. This led to an almost null survival rate, similarly to what can be observed on sandstone or coral skeleton tiles (Harrington et al. 2004; Burt et al. 2009). There is a need for a compromise between durability and environmental impact of any material. For example, ceramic and clay might be more inert and produce less microplastic pollution than PVC, but they would not last long-term in seawater.

Out of all our tested materials, PLA with flax fibers might be the best compromise for settlement, survival, durability, lower competition, and renewable resources. Nonetheless, recent experiments have tested various innovative materials that could offer similar or better results. Studies testing the efficacy of 3D printed clay have shown promising results (Berman et al. 2023b). Sand-magnesia mortar can also show the same success as PVC for coral settlement (Suzuki et al. 2020). Future experiments comparing the most promising innovative materials to each other for the long-term coral recruitment should be encouraged.

As mentioned before, future efforts should focus on combining restoration practices. For example, corals should not be transplanted at random or bred haphazardly, but the genetic potential of certain genotypes to restore an area or to transmit their specific resistance to their offspring should be taken into consideration (Randall et al. 2020). Proactive coral restoration should not simply aim to restore an ecosystem to a former state, but take climate predictions into account (Caruso et al. 2021). An easy way for restoration practitioners to grow out heat-resistant corals is to sample colonies that survived in sites which showed high mortality rates during bleaching events, or colonies that are naturally adapted to extreme heat (Caruso et al. 2021), then multiply those samples in nurseries before outplanting. Alternatively, using a large number of different genotypes from various origins can capture a large diversity that is more likely to withstand a combination of disturbances than just a few selected genotypes (Baums et al. 2019).

The restoration community would also benefit from improved communication between restoration projects (Boström-Einarsson et al. 2020a). For example, the coral restoration consortium aims to regroup all results from hundreds of restoration projects all around the world (CRC 2023), and the ICRS' coral list can be a good tool to connect scientists and practitioners. Generally, we need more comparable research in remote areas, in low-income countries and in areas lacking governmental support (Reimer et al. 2024). Open access data

also greatly improves comparisons and the spread of information. For this reason, data and scripts used for this PhD are available on Github and finished papers will be accessible on ResearchGate, which are great open access tools to inform people working in the same field.

## Limitations of this PhD

As a PhD thesis is never completed without a series of mistakes to learn from, some limitations of the presented experiments should be addressed. The relatively short monitoring of experiments between 2020 and 2022 might not allow the results to be extrapolated to the future environmental conditions. Also, a lack of spatial replicates could be noted in our settlement experiments on artificial materials. If there were a strong site effect on settlement patterns, this could not have been noticed in the presented experiments. Thus, the replicability of these results to other sites in Mo'orea or Polynesia remain hypothetical. There can actually be strong site effects in settlement patterns and in survival of recruits depending on biotic or abiotic environmental conditions (Sherman et al. 2001; Burt et al. 2009; Ferse et al. 2013). For example, on the western fore reef of Mo'orea, recruitment rates can be much lower than on the North shore because of a higher exposure to waves (Adjeroud et al. 2007). The coral cover of a site can also significantly influence the recruitment patterns, as adult *Acropora* cover can increase acroporid recruitment, but the opposite can be true for pocilloporids (Penin et al. 2010; Bramanti & Edmunds 2016).

To improve the gathered data, after the last monitoring of settlement tiles, the recruits could have been sacrificed to be identified to their family and potentially reveal family-dependent survival patterns. Another simple way to add resolution to our monitoring of nursery and outplanted corals in chapters 2 and 3 would have been to add a color scale to each photo (Marshall et al. 2012; Knipp et al. 2020). This would have allowed to measure the evolution of pigmentation in corals in addition to monitoring their growth, which could have been a good indication of the health state of our colonies. The 2D photos taken from above allowed to measure a certain growth, however the vertical growth was omitted with this technique. This could have been improved by monitoring coral growth in 3D through photogrammetry techniques. In general, all experiments would have benefited from a more careful planning and complete protocol, for instance to have a more consistent biotic (including macroalgal and fish surveys) and abiotic monitoring of environmental conditions in parallel to the measurements taken on our coral colonies.

Considering all surviving nursery corals during the 2019 bleaching presented an improved thermal tolerance relative to the dead ones, our work could have aimed towards deciphering the causes behind these differences, either by comparing the genetic material of resistant and sensitive corals, or later by measuring the thermal resistance of colonies through ex situ experiments. This could have allowed to pinpoint whether the resistance was maintained even after two years of nursery rearing or after outplanting, and whether it was significantly higher than in wild populations that were less impacted by the 2019 heat wave. Due to a lack of time

and means, these types of experiments could not be performed during the current PhD but could be considered for further improving our understanding of coral resistance in the future.

## Actual benefits of coral restoration, or hopeless drop of water in the ocean?

The growing threats on coral reefs have sparked an impressive surge in coral restoration research (Boström-Einarsson et al. 2020a). The perspective of coral restoration sparks a lot of interest because of the hope it gives. In fact, the best way to provoke the interest of the public is to send a message of hope. Pessimistic news tends to lead people into a catatonic state of despair, while the possibility of hope is more likely to induce actions. Thus, private investors for innovative restoration projects are easily enthused, not to the least by the prospect to offset their ecological impact by investing in the restoration of endangered ecosystems (Suggett et al. 2023). There is thus a risk to mistake a greenwashing project selling hope to the public by an actual successful restoration project. Terms like “super corals”, “bright spots” or even “assisted evolution” can spark a lot of enthusiasm but need to be carefully defined in order to send a scientifically correct message.

There is a certain bipolarity among many coral scientists, who are aware of the almost inevitable fate of coral reefs and still try to sell a message of hope and avoid the “doom and gloom” message (Braverman 2016). Putting hope in restoration may seem naïve, but persisting with coral research is near impossible if we believe that all hope is lost and do not keep a certain optimism. The very existence of coral reef ecosystems being at stake, Reimer et al conclude their paper on the current fourth global bleaching by stating: “Our ability to limit damage from excessive emissions still exists. We know most of the solutions and implementation is urgent” (Reimer et al. 2024). Or as Nancy Knowlton tweeted: “Bleaching is bad but giving up is worse”, as part of the Ocean Optimism movement (Knowlton 2016). Nonetheless, coral restoration can be a divisive subject among coral scientists. “Restoration is crap “ says Andrew Baird in (Braverman 2016), inferring that restoration is taking over research interest and funding while coral ecology is left behind. “I’m not saying that we shouldn’t be trying to refine the techniques, but until we deal with the climate issue, this is futile.” says Ove Hoegh-Guldberg in (Braverman 2016). Tackling the climate issue thus seems an utmost priority relative to restoration efforts (Anthony et al. 2020).

Similarly, climate mitigation can induce a lot of controversy in politics. “The Australian government is trying to prolong the export of coal. Obviously, the last thing the Great Barrier Reef needs is more coal mines” says Terry Hughes in (Braverman 2016). The Australian government actually censored the UNESCO’s 2016 climate change report linking the degradation of the Great Barrier Reef to GHG emissions (Readfearn 2016). This nicely illustrates the conflict of interests between the pursuit of growth and profit and the plead to protect natural ecosystems while there is still time.

> "The restoration of the planet is the greatest challenge of our century, of our lifetime even. If we don't work on it, there is no future. And I'm not talking about the future for our children, because I don't think as a species we really care about our children, or we wouldn't be behaving as we are. So, I would suggest just conserve and restore for yourself and your lifetime. You need to do the restoration to jump start nature because nature is so far gone it's not going to do it by itself" says Sarah Frias-Torres in (Braverman 2016).

With the NOAA and IPCC predictions, the end of ocean warming and bleaching events is not yet in sight (Hoegh-Guldberg et al. 2018). Bleaching events are predicted to increase in frequency and severity (Hughes et al. 2017b), as shown during the current Fourth Global Coral Bleaching Event (Reimer et al. 2024). Thus, simple restoration projects where corals grow for five or ten years then bleach and die are futile. We need more research to better understand how coral can cope to future conditions (Madin et al. 2023). In parallel, serious management of pollution and fishing pressures should be implemented all over the world, following the models of Bonaire or Bermuda where fish traps have been banned to protect herbivore populations (Jackson & Johnson 2014; Steneck et al. 2019).

It is true that coral restoration techniques are very costly, the most expensive out of all marine restoration (Saunders et al. 2020), and labor-intensive for very little positive outcome. Of course, some restoration efforts demonstrate undeniable benefits. For example, they can significantly improve the coral cover, but on very localized zones (Fox et al. 2019; Williams et al. 2019; Lange et al. 2024). After just four years, carbonate budgets in the restored site were the same as in healthy control sites (Lange et al. 2024). This can potentially enhance the genetic diversity and favor sexual reproduction for many years, increasing the recovery of the greater area, assuming only that no major disturbance causes the destruction of the restored reef in the meantime. In fact, farmed or transplanted corals are not immune to local and global disturbances, and years of work can be wiped out by a single bleaching event (Fadli et al. 2012). If climate change, pollution, overfishing, the spread of diseases and invasive species are not controlled, the life expectancy of restored coral reefs is seriously compromised.

Long-term, restoration efforts cannot stop the looming threat of climate change. This is why the link between humans, coral reefs and a warming climate needs to be at the center of our work and discourse. With our past and predicted carbon emissions, coral reefs as we know them have almost no chance to acclimate to the rapidly changing conditions. If climate change is not addressed with absolute urgency, coral islands will disappear under rising seas, coastal cities will lose their protection against swells and storms, and their fishing resources. The decline of coral reefs is only one of the reasons why reducing our carbon emissions is quite literally a matter of life and death.

## Sociocultural and economic impacts of coral restoration

Nonetheless, if failing at increasing coral cover and resilience durably, restoration projects can turn out to be beneficial for different reasons, notably socioeconomic aspects. While not directly addressed in this PhD thesis, sociocultural and economic aspects of any projects should be taken into account, as together with environmental impacts, they contribute to the sustainability of any project, and are thus inherent to the resilience of coral reefs (Hein et al. 2017). The four pillars of sustainability can be summarized by environmental, sociocultural, governance and economic contributions (Hein et al. 2017).

Restoration projects can involve many local volunteers, create paid jobs and increase tourism, boosting the economy even in remote islands (Fadli et al. 2012; Hein et al. 2019; Saunders et al. 2020). Cultural ecosystem services, such as aesthetic, recreational and educational services are linked to wellbeing and a better social cohesion (Hein et al. 2017). While scientific communication and citizen science are important, a direct contact between the public and corals can show the best results for raising awareness and engagement (Hesley et al. 2017). Similarly, marine protected areas perform better when local communities are included in their design and implementation (Ferse et al. 2010). By involving as many people as possible through visits of restoration facilities, interventions in schools and other outreach and education efforts, the general public becomes aware of the importance of coral reefs and the threats they are facing. I had the chance to witness this myself through regular exchanges with the inhabitants of Mo'orea, with numerous visits to school classes in four different islands, and exchanges with people wanting to get involved in coral research or restoration. By promoting involvement of local volunteers and education of the younger generation, scientists can effectively promote coral reef conservation stewardship, which is invaluable for raising awareness and demanding better protection and management of coral reefs. There is in fact a 'governance crisis' associated with the decline of coral reefs worldwide (Hughes et al. 2010). A better awareness and involvement of the general public is essential for enhancing engagement and collectively acting towards changing laws harming us and coral reefs. Ultimately, collective action between scientists, the general public and policy makers is our best hope for coordinating efforts in improving the conditions for coral reefs.

Sharing cultural and traditional values helps respect the local customs to ensure that a project can be fully accepted. This can prevent problems associated with parachute science or colonial science, the common process by which scientists come to an unfamiliar island, collect their data then leave without sharing their intentions or results (Stefanoudis et al. 2021; Odeny & Bosurgi 2022). Thankfully, projects are increasingly aware of the social impacts they can have and make sure to not contribute to injustices or inequalities.

## Future directions in research

Despite the rapid advances, the most promising coral restoration techniques are still at the experimental stage. Thus, a lot more research needs to be undertaken to improve the scalability of coral restoration. Also, long-term monitoring is crucial to fully appreciate the

recovery of a restored site to a new state of equilibrium, to account for the socio-economic benefits and to identify the most cost-effective methods.

As heat stress is currently the most urgent threat to coral reefs, more research is needed to either improve heat resistance of sexually produced offspring by hybridizing different species (Chan et al. 2018), different populations (Quigley et al. 2020; Howells et al. 2021), pre-exposing them to high temperatures (Puisay et al. 2023), or combining them with heat-resistant symbionts (Quigley et al. 2023). For instance, we found in chapter 4 that by specifically selecting compatible parents, the fertilization rate at 31°C, which is 4°C above the seasonal average, could be 91-fold increased relative to the least compatible parents. This could be promising for future restoration efforts aiming to produce large quantities of heat-resistant larvae. By seeding these larvae on degraded reefs, they could potentially improve the resilience of heat-sensitive populations by combining their genetic material with that of local corals. Future experiments should focus on producing descendants with the most successful pairs of genotypes at high temperatures to determine if their thermal resistance can be maintained at the larval or recruit stage.

More precise identification methods using genetics to discriminate between coral species would additionally benefit coral research. In fact, many species considered common are actually composed of species complexes, such as *Pocillopora verrucosa* or *Acropora hyacinthus*, that cannot be easily identified visually (Suzuki et al. 2016; Johnston et al. 2022). More investments in improving the genetic markers for discriminating between species could improve our understanding of which species are common and which are threatened.

Other new techniques are seeing increased use in coral research. Machine learning could have increased the efficacy of photoquadrat analysis in my PhD. In fact, for chapter 5, 6 and 7, 1400 individual photos of settlement tiles were identified by hand on 49 points for their benthic communities, sometimes more than once if misidentifications were noticed. This time-consuming process could have been greatly accelerated through an automatic software able to recognize the main benthic categories. As this tool was not available at the start of the PhD, all analysis were done by hand, which can prove to yield more accurate results, but are very time-consuming and should be done by a single operator so as to not introduce identification biases. Machine learning is now more commonly used in coral research (Burns et al. 2022), but its accuracy and availability would benefit from increased research effort and data collection.

We need greater research efforts in the scalability of restoration practices, as a greater scalability would reduce failure rates and allow the expansion of restored areas. In fact, while artificial structures combined with transplantation can show promising results on small scales, it is near impossible to spread these interventions to the whole ecosystem. Industrial-scale interventions are technically feasible but require a substantial investment. Manual COTS control is already done at an industrial scale in Australia, at a substantial cost of AUD 25 million/year, and viewed as an effective way to increase the long-term coral cover (Westcott

et al. 2020; Castro-Sanguino et al. 2023). Large-scale larval seeding projects would require a helicopter and a tugboat with a pump and large tanks to collect spawn slick to be translocated (Doropoulos et al. 2019b, 2019a). This method was estimated to produce three times more mature colonies after four years, each valued at USD 55, than the transplantation of ten thousand mature colonies, costing each USD 206 after four years (Doropoulos et al. 2019a). It also has a lower impact on native coral populations.

Various large-scale applications aiming to reduce bleaching severity are also being researched. The deployment of a surface film, made out of calcium carbonate, or of microbubbles through a surfactant could attenuate 20% of light and prevent some bleaching over high-value reefs (Bay et al. 2019). However, the stability and durability of these films on circulating seawater would be very low. Cloud brightening or cloud seeding by spraying seawater could also reduce the severity of heat waves by enhancing the albedo on a larger scale, thus also reducing coral bleaching rates (Latham et al. 2013; Ahlm et al. 2017; Bay et al. 2019). Unfortunately, a lack of risk assessments on rainfall modification and ethical concerns on geoengineering still seem to delay the deployment of this promising technology (Bay et al. 2019).

Restoration should be thought of not only at the coral reef scale, but include other habitats to restore, which can facilitate the recovery of reefs through interactive processes (Vozzo et al. 2023). Cross-habitat facilitation can happen when mangroves are protected for their ability to trap sediments which in turn improves the clarity of neighboring coral reef water (Vozzo et al. 2023), or when the protection of seabird nesting sites increases the nutrient input that favors coral growth (Savage 2019). Small-scale restoration efforts should slowly be replaced by global projects impacting much larger scales with scientific validation, in line with the UN's Decade on Ecosystem Restoration objectives.

## Conclusion

With the threats resting on coral reefs, a lot of research has been aimed at enhancing the outcomes of restoration practices. Significant progress has been made in coral research, and with important scientific communication and media coverage, coral restoration has become well understood by the general public, at least by populations living and holidaying near coral reefs (Le et al. 2022; Ochieng et al. 2024). However, there are still many unknowns in coral research, and many promising methods are still at the experimental stage. Assisted evolution, for instance, by increasing the thermal resistance of many different coral species and spreading them to as many regions as possible would be a way to rapidly increase the resilience of endangered reefs (Chakravarti & van Oppen 2018; Howells et al. 2022).

As more and more coral nurseries are built around the world, a better understanding of the growth, physiology, and reproduction of corals within these nurseries is important to optimize their success. Similarly, as private and public investors increasingly plan to build artificial reefs as ecological compensation, a boost of tourism or fisheries or for coral restoration, it is important to provide engineers and material scientist with a detailed appreciation on how

artificial materials will influence the first life stages and the long-term fitness of recruiting corals. The results of the present PhD thesis will thus hopefully contribute positively to the global expertise on both asexual and sexual restoration practices. Unfortunately, as all coral reefs are currently critically threatened, restoration practices can seem like futile efforts for very little positive outcome. One of the most important areas to improve on would be the scalability and long-term outcome of restoration projects. Letting go of the ineffective projects and focusing collective efforts on the actual valuable strategies would greatly benefit the conservation of corals. Of course, restoration efforts will be vain if climate change is not fought with an absolute urgency. If it is not fought for the sake of corals, then at least for the invaluable ecosystem services they grant us and our future generations.

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## Résumé substantiel (summary in French)

Les récifs coralliens tropicaux sont des écosystèmes inestimables grâce aux nombreux services écosystémiques qu'ils prodiguent. D'une part, ils présentent par unité de surface une biodiversité inégalée par d'autres écosystèmes. Cette abondance d'espèces est indispensable pour l'approvisionnement de nombreuses populations côtières en nourriture et matériaux de construction, et plus récemment pour l'industrie pharmaceutique qui révèle régulièrement de nouveaux composés bioactifs prometteurs à partir d'organismes récifaux. Si les récifs coralliens bénéficient aussi au secteur touristique en attirant chaque année 70 millions de touristes, leur service le plus crucial est la protection côtière. En effet, les barrières de corail protègent les côtes des tempêtes, tsunamis et fortes houles, et permettent à 200 millions de personnes de vivre à proximité ou au niveau de la mer.

Les récifs coralliens sont formés par des coraux scléractiniaires hermatypiques, qui sont généralement des animaux coloniaux sessiles formés de milliers de polypes tous génétiquement identiques. Une endosymbiose avec une microalgue photosynthétique de la famille des Symbiodiniaceae leur permet de prospérer dans des eaux tropicales très pauvres en nutriments. C'est lorsque cette microalgue pigmentée est expulsée lors de fortes chaleurs que les coraux blanchissent et finissent souvent par mourir de faim.

Les coraux présentent de nombreux modes de reproduction différents, asexuellement par bourgeonnement, fragmentation ou parthénogénèse et sexuellement par production de gamètes, qui sont soit fécondés en pleine eau ou à l'intérieur du polype. Les larves formées après quelques jours sont capables de nager sur des kilomètres avant de se fixer sur un substrat qui leur convient. Afin de choisir ce dernier, les larves sont capables de se diriger vers des sons d'un récif en bonne santé, vers les molécules produites par des congénères, vers les couleurs des algues favorisant leur fixation tout en évitant les fortes luminosités et les molécules produites par des organismes compétiteurs. Certaines algues calcaires ou CCA (crustose coralline algae) favorisent la fixation et la survie des juvéniles, appelés recrues, alors que les macroalgues, éponges ou bryozoaires vont diminuer leur survie en les recouvrant. La prédation par les poissons brouteurs va également réduire la densité en recrues sur des surfaces exposées. C'est pour cette raison que les larves se fixent en préférence dans des abris cryptiques.

Malgré leur rôle clé dans l'écosystème, un tiers des coraux est actuellement menacé d'extinction et le recouvrement corallien global a diminué de moitié depuis 1950. Les menaces naturelles sur les récifs coralliens sont les tempêtes et invasions d'acanthaster, un prédateur de corail vorace. Les menaces anthropiques causant des dégâts directs sont les constructions côtières, la pêche à la dynamite et l'ancrage des bateaux. La pollution venant des villes côtières, de l'agriculture ou des bateaux de croisière augmente la prolifération d'algues qui entrent en compétition avec les coraux. Ensuite, la surpêche de poissons herbivores empêche la régulation des populations d'algues et le rétablissement des coraux. Pour ces raisons, les récifs coralliens vont vers une dominance d'algues au détriment des coraux. La pire menace

reste néanmoins le réchauffement climatique, car un seul épisode prolongé de chaleur anormale peut causer la mort de tout un récif, si celui-ci est composé de coraux thermosensibles. Au vu des prévisions du GIEC et de tous les spécialistes du sujet, les récifs coralliens actuels sont voués à disparaître dans les 25 prochaines années. Il est donc crucial de réduire au maximum nos émissions de gaz à effet de serre, en changeant notre système basé sur la croissance et la consommation, si l'on veut continuer de profiter des services fournis par les coraux.

En conséquence des menaces croissantes sur les coraux, de nombreuses initiatives de restauration corallienne ont vu le jour au cours des 30 dernières années. Ces mesures visent à augmenter le recouvrement corallien, la diversité génétique ou la résilience des coraux face aux futures perturbations. La méthode la plus courante est le bouturage et repiquage de fragments de coraux pour restaurer des sites à faible diversité ou qui ne reçoivent plus de larves de coraux naturellement. Idéalement, la transplantation se fait après un élevage en pépinière, pour limiter les prélèvements sur des colonies sauvages. Cette pépinière permet de multiplier asexuellement de nombreuses petites boutures et de les laisser grandir dans des conditions propices pendant 4-24 mois, selon leur vitesse de croissance, avant de les transplanter.

Seulement quatre pays regroupent à eux seuls 40% des projets de restauration, et trois espèces de coraux branchus sont majoritairement utilisés en restauration. Les autres régions et espèces moins communes sont donc largement sous-représentées dans les études sur le sujet. Alors que la survie et la croissance sont les paramètres les plus suivis, la reproduction sexuée des coraux restaurés n'est que très rarement rapportée, probablement à cause de la courte durée de suivi de la plupart des projets de restauration. Pourtant, la reproduction des coraux plantés peut être un bon indicateur de renouvellement et de succès de restauration.

Le style de pépinière permettant la croissance la plus importante est la pépinière suspendue, où les coraux sont accrochés à des fils de pêche. Sans support fixe et profitant d'un bon brassage d'eau, les coraux peuvent grandir trois fois plus vite suspendus que attachés à un support. Le site choisi pour une pépinière est également important pour le succès de celle-ci. Par exemple, un lagon peu profond peut être bénéfique pour la protection des intempéries et un rayonnement solaire important, mais peut être sujet à plus de pollution, turbidité ou sédimentation qu'un site plus profond côté océan. Néanmoins, l'influence des paramètres de l'habitat sur la santé des coraux en pépinière reste peu étudiée.

La première expérimentation de ma thèse, détaillée dans le chapitre 2, avait pour but d'étudier l'impact de la translocation d'une pépinière de lagon de cinq mètres de profondeur vers un site en pente externe à 14 mètres de profondeur sur la santé des colonies coralliennes. La survie, la croissance et maturité de cinq espèces d'*Acropora* ont été suivis dans les deux sites pendant un an. L'acclimatation physiologique a été étudiée sur une espèce modèle. Nos hypothèses étaient que la survie serait constante, mais que la croissance et maturité

pourraient être limités en pente externe du fait de la quantité limitée en lumière, donc en énergie disponible.

La pépinière lagon est composée de 30 arbres à coraux, des structures verticales ancrées dans le sable et flottant grâce à une bouée submergée. Le long du tronc, 20 branches permettent de suspendre 60 fragments de coraux à des fils de pêche. Sur la pente externe, dix arbres ont été fixés à des anneaux vissés dans la roche à 14 mètres de profondeur. Des fragments de coraux de cinq espèces d'*Acropora*, *A. hyacinthus*, *A. globiceps*, *A. nasuta*, *A. retusa* et *A. striata,* qui grandissaient en pépinière lagon depuis au moins six mois, ont été sélectionnés. Pour chaque génotype (entre 7 et 25 selon les espèces), trois réplicas ont été gardés en pépinière lagon, et trois ont été déplacés vers la pente externe. Leur survie, croissance et maturité ont été évaluées régulièrement. Pour *A. hyacinthus,* un échantillon de 3 cm a été prélevé avant et un an après translocation pour évaluer l'acclimatation physiologique. La concentration en Symbiodiniaceae et en chlorophylle *a* et $c_2$ ont été mesurés en fonction de la surface du fragment.

Avec en moyenne une variation journalière de 0.26°C, les températures en pente externe étaient moins variables qu'en lagon (1.12°C d'écart journalier), et la luminosité atténuée de moitié en pente externe. Alors que presque aucune mortalité n'a été observée en un an, la maturité et la croissance de nos coraux étaient plus influencées par la taille et l'espèce que par l'habitat. En effet, les fragments de petite taille (< 50 cm²) grandissaient proportionnellement plus que les plus grands, alors que les grands (> 100 cm²) avaient plus de chances d'atteindre la maturité. En revanche, il y avait une interaction significative avec l'espèce : pour une même taille, *A. nasuta* avait le plus de chance d'être mature, suivi d'*A. retusa*, *A. hyacinthus*, *A. striata* et *A. globiceps*. Cependant, la croissance la plus rapide était observée chez *A. nasuta*, suivi de *A. striata* puis *A. hyacinthus* et *A. retusa*.

Après la taille et l'espèce, l'habitat avait également un effet significatif : en lagon, les coraux, en particulier *A. hyacinthus*, avait un plus haut taux de maturité qu'en pente externe. Par contre en pente externe, la croissance était soit identique, soit plus élevée chez *A. nasuta* et *A. globiceps*, ce qui était contraire à notre hypothèse de base. En prenant en compte la physiologie des *A. hyacinthus*, nous avons observé que la densité en symbiontes était le plus corrélée à leur croissance, en interaction avec l'habitat et la chlorophylle. Curieusement, la densité en symbiontes chez *A. hyacinthus* était plus élevée en lagon. C'est donc en augmentant significativement sa concentration en chlorophylle *a* par symbionte en profondeur que le corail était capable de maintenir une croissance similaire dans les deux habitats.

Cette expérience révèle que les pépinières peuvent présenter un succès similaire en lagon qu'en pente externe, grâce à une acclimatation physiologique rapide des coraux à leur nouveau milieu. La pente externe présente certains avantages, comme des températures plus stables, de nombreux poissons herbivores et une absence d'algues flottantes qui dégradent les coraux en lagon. Il est aussi possible que les 14 m de profondeur ne limitent pas assez la luminosité pour réduire significativement la croissance des coraux. En revanche, les pépinières en pente

externe sont plus sujettes aux dégradations causées par la forte houle qu'en lagon. *A. nasuta* et *A. hyacinthus*, grâce à leur croissance et maturité élevées, sont de bons candidats pour optimiser le rendement des pépinières dans le futur. Des expériences complémentaires pourraient déterminer si le compromis entre la croissance et la maturité persiste après un an, et si les différences liées aux habitats sont également observées chez d'autres genres.

Après avoir atteint une taille de plus de 10 cm chez les coraux branchus ou 5 cm chez les coraux massifs ou encroûtants, les coraux de pépinières peuvent être utilisés pour des projets de transplantation. Cela sert à restaurer des sites endommagés en augmentant le recouvrement corallien donc également la biodiversité associée et l'attraction de nouvelles recrues, et en augmentant la diversité génétique de certaines populations menacées, permettant donc d'augmenter leur résilience face aux futures perturbations. La survie, croissance et reproduction des coraux transplantés vont dépendre de nombreux facteurs, notamment la taille, le génotype ou le microbiome du corail, les paramètres physico-chimiques du site, la compétition et la prédation.

Le chapitre 3 de ma thèse étudie le succès de transplantation d'*Acropora cytherea* élevés en pépinière. Cette espèce forme de grands plateaux en lagons en Polynésie, mais devient rare dans certains sites et n'est quasiment pas représentée dans les études de restauration corallienne. En choisissant trois sites de transplantation qui présentaient tous des températures, recouvrements coralliens et algaux et des degrés de dégradations et de pollutions différents, notre hypothèse était que le site de référence, donc présentant le plus de coraux en bonne santé, aurait le plus de succès pour la transplantation. Nous avons également supposé que différentes communautés symbiotiques pourraient favoriser la croissance et la survie des coraux, mais qu'elles évolueraient en fonction du temps et du site. Les effets génotype, taille et symbiontes étaient présumés avoir moins d'impact que l'effet site.

Après avoir grandi pendant deux ans en pépinière en lagon, qui est décrite plus haut, 12 réplicas de 8 génotypes différents d'*A. cytherea* ont été sélectionnés pour être soit transplantés dans un des trois sites, soit pour rester en tant que contrôle dans la pépinière lagon. Leur survie, croissance et maturité ont été suivies pendant plus de deux ans dans les quatre sites. L'ADN de petits fragments prélevés avant, après 12 et 27 mois a été extrait pour identifier les différents groupes de symbiontes présents dans chaque colonie.

Le site de référence, Linareva, était caractérisé par des diversité et recouvrement élevés en corail dont des populations sauvages d'*A. cytherea*, beaucoup de macroalgues et peu d'azote. Le site le plus impacté par la présence humaine, Manava, présentait un fort taux en azote, une température stable, un recouvrement algal fort et peu de corail vivant. Le site intermédiaire, Mahana, avait un peu plus de corail vivant, peu de macroalgues et d'azote, et une température variable. Malgré ces conditions très distinctes, le site de transplantation n'avait pas d'effet significatif sur la survie, la croissance globale ou la maturité des fragments transplantés, contrairement à notre hypothèse initiale. C'était plutôt la taille qui influençait le plus la survie et la maturité, avec les grandes colonies présentant significativement plus de chances de survie

et de maturité. La survie était plus élevée les quatre premiers mois que les dix derniers mois, et la maturité a fortement augmenté la deuxième année (49%).

La croissance dépendait surtout de l'état de santé du corail, puis d'une interaction temps et site, avec initialement les colonies à Mahana et Manava qui diminuaient en taille plus qu'à Linareva. Ceci peut s'expliquer par une prédation par des poissons plus intense dans ces deux sites. Après huit mois, il n'y avait plus de différence de croissance entre sites, et après un an, la croissance est devenue positive. Une diminution de croissance initiale peut aussi s'expliquer par le stress de la transplantation, des conditions défavorables, de la compétition algale et une nécessité d'augmenter la densité squelettique avant d'augmenter l'extension linéaire. En effet, les coraux en pépinière sont suspendus et peuvent donc avoir un squelette plus léger et fragile et grandir plus rapidement, contrairement aux coraux transplantés soumis à la pression du courant et la prédation.

Alors que chaque génotype présentait des communautés en symbiontes distinctes, il y avait une tendance graduelle à perdre le genre *Durusdinium*, qui est lié à une meilleure tolérance thermique, en faveur du genre *Cladocopium*, qui était corrélé à une meilleure survie et croissance. Ceci peut être dû à l'absence de stress thermique observé durant l'expérimentation. Cependant, il n'y avait pas de communautés symbiotiques liées à un site de transplantation ou un autre.

L'absence d'effet site est une bonne nouvelle, car elle révèle que la transplantation peut présenter autant de succès dans un site considéré sain que dans un site plus impacté. Cela permettra d'avoir des transplants survivants et présentant une bonne croissance qui pourront à terme restaurer des sites dégradés. Bien sûr, les menaces locales comme la pollution et la surpêche doivent être contrôlées en parallèle des efforts de transplantations pour avoir de bons résultats.

La transplantation de coraux adultes est un travail laborieux et coûteux. Vu qu'elle est basée sur la reproduction asexuée, elle ne produit pas de nouveaux génotypes. Il est pourtant important de prendre en compte la diversité génétique d'une population pour estimer sa capacité à résister à des perturbations. Si la restauration se fait sur base de très peu de génotypes différents, cela limite la diversité génétique. En revanche, la restauration basée sur la reproduction sexuée des coraux permet de créer des milliers de nouveaux génotypes rapidement. Les gamètes de coraux peuvent être récoltés sur le terrain ou en laboratoire, fécondés, et une fois les larves compétentes, elles sont utilisées pour coloniser des petits substrats artificiels ou directement des portions de récifs dégradés. Les taux de survie peuvent dépasser ceux de coraux adultes transplantés, ce qui rend la propagation de larves ou de recrues beaucoup plus rentable du fait des travail et coût réduits. D'autres techniques complémentaires comme l'hybridation, les croisements sélectifs ou la préexposition des gamètes à des conditions défavorable pourraient en théorie augmenter le succès des projets de réensemencement.

Très peu d'études ont été réalisées sur la restauration utilisant la reproduction sexuée des coraux. Par exemple, seulement neuf papiers ont testé l'ensemencement in situ de zones naturelles dégradées, mais montrent des résultats très prometteurs. Aussi, très peu d'études se sont penchées sur l'effet parental lors de la fécondation, et si celle-ci peut être améliorée en choisissant certains couples plutôt que d'autres. Afin de combiner la restauration utilisant la reproduction sexuée et asexuée, les coraux élevés en pépinière pourraient servir à fournir des descendants pour augmenter le rendement de la restauration. Ceci pourrait également éviter la récolte et l'endommagement d'individus sauvages afin de récolter des larves. Cependant, seulement deux études ont comparé la capacité reproductive de coraux en pépinière à celle de populations sauvages, et ont souligné leur potentiel à produire significativement plus de descendant que les populations sauvages du fait de la densité élevée en coraux interféconds.

Le chapitre 4 de ma thèse est basé sur l'hypothèse que la capacité reproductive des coraux élevés en pépinière serait la même que celle de coraux sauvages. Cela est important à vérifier si les pépinières doivent fournir des coraux qui repeupleront des sites endommagés et devront s'intégrer dans les populations hôtes. Un autre objectif était de tester si les taux de fécondations pouvaient être optimisés même à température élevée en croisant certains génotypes de la pépinière. Enfin, nous avons émis l'hypothèse que l'ensemencement de larves obtenues lors des pontes en laboratoire pourrait significativement augmenter les taux de recrutement de sites dégradés du lagon, même en utilisant des outils simples et communs. Ces tests visent à améliorer les projets de restauration combinant les techniques sexuées et asexuées et suggérer des pratiques de restauration efficaces et accessibles à toute une panoplie de prestataires.

Quelques jours avant la ponte présumée, des colonies de cinq espèces d'*Acropora*, *A. pulchra*, *A. hyacinthus*, *A. cytherea*, *A. nasuta* et *A. retusa,* ont été récoltées à la pépinière en lagon décrite plus haut, et dans quatre sites autour de Mo'orea, et ramenés en aquarium au centre de recherche. Leur fécondité, c'est-à-dire le nombre d'œufs par cm² de tissu et par bundle (paquet de gamètes), la taille des œufs et le taux de fécondation à température ambiante et élevée ont été mesurés. Les populations de la pépinière présentaient la même heure et jours de ponte que les populations sauvages, sauf pour deux espèces lagonaires où la ponte était retardée de deux jours chez la population en pépinière. Comme ce retard n'était plus observée lors de la troisième saison de ponte, il aurait pu être causé par le stress de la manipulation, du transport ou du milieu ex situ. La fécondité ne variait pas selon les populations ou l'espèce, et la taille des œufs variait seulement selon les espèces. Le nombre d'œufs par bundle et le taux de fécondation variaient surtout en fonction des espèces, et seulement les *A. retusa* de la pépinière avaient des valeurs inférieures à celles de la population sauvage. Ceci pourrait être expliqué par la petite taille de ces colonies à la pépinière comparé à celles des colonies sauvages. Pour les autres espèces, la capacité reproductive est la même en pépinière ou en milieu naturel. Ceci permettra aux coraux de la pépinière d'intégrer et se reproduire avec des

populations sauvages après transplantation, et ainsi participer à augmenter leur diversité génétique et leur résilience.

En croisant huit génotypes différents d'*A. cytherea*, nous avons observé que les taux de fécondation pouvaient être améliorés 13 fois à 27°C ou 91 fois à 31°C en choisissant les parents les plus efficaces. Même si la fécondation à 27°C présente quatre fois plus de succès que celle à 31°C, certains génotypes semblent produire de bonnes quantités d'embryons viables malgré un fort stress thermique. Ceux-ci pourraient être utilisés dans le futur pour produire des descendants ayant une thermo-résistance élevée. Curieusement, les génotypes les plus compatibles présentaient également des communautés symbiotiques similaires. Nous avons aussi trouvé que les genres *Symbiodinium* et *Cladocopium* étaient positivement corrélés au taux de fécondation, contrairement au genre *Durusdinium*. *Durusdinium* est habituellement associé à une tolérance thermique élevée chez les adultes, mais peut en retour compromettre la croissance et l'investissement reproductif.

Avec les milliers de larves obtenus après les pontes en aquarium, les larves d'*A. cytherea* ont été choisies pour ensemencer des patates mortes (corail massif mort) du lagon de Mahana. Six filets en forme de tente ronde, de 1.2 m de diamètre ont été disposés au-dessus de ces patates. Avec un tuyau et un entonnoir, les filets ont été remplis depuis la surface avec 12'000 larves chacun. Après dix jours, aucune recrue naturelle n'était fixée sur les six patates contrôles, mais 3.5 recrues par 100 cm² ont été comptées sur les patates ensemencées. Après un an, cette densité est tombée à une moyenne de 0.11 recrue par 100 cm², mais toujours aucune larve ne s'était fixée sur les patates contrôle, confirmant le taux de recrutement naturel extrêmement faible dans le lagon de Mo'orea. Avec des outils très communs et relativement peu d'effort, nous avons démontré qu'il était possible de significativement augmenter le taux de recrutement de zones à restaurer. En fonction du taux de survie à long terme, cette technique pourra se montrer plus rentable que la transplantation de coraux adultes, qui demande beaucoup plus de travail de bouturage, d'élevage et d'entretien.

Différentes techniques de restauration pourraient encore améliorer la survie de recrues coralliennes. Pour augmenter les surfaces à coloniser et la survie des recrues en leur offrant des refuges, la création de récifs artificiels adaptés peut également contribuer à la restauration d'un site dégradé. Ces structures peuvent présenter toutes formes ou textures, mais sont généralement faites en béton, et servaient initialement à augmenter la densité en poissons pour améliorer le succès de la pêche. Depuis l'intérêt grandissant pour la protection des coraux, les récifs artificiels sont également étudiés pour leur capacité à améliorer le recouvrement corallien. Alors que beaucoup de matériaux ont été testés lors d'études de recrutement, les connaissances sur les influences des matériaux, textures et tailles des trous sur la densité, la survie et la croissance des recrues sont encore assez vagues, en particulier lorsqu'on ajoute l'influence des autres communautés d'organismes sessiles qui s'y installent.

Les chapitres 5, 6 et 7 explorent le potentiel des matériaux et textures innovants pour augmenter le taux de recrutement corallien naturel, ainsi que la diversité, survie, et croissance

de coraux juvéniles. La première expérience, détaillée dans le chapitre 5, compare l'efficacité de huit matériaux innovants et deux matériaux communs en tant que substrat de recrutement corallien. Le chapitre 7 est un réplica temporel de cette expérience, avec en plus un suivi à long terme pour pouvoir suivre la croissance et la survie des premières recrues après 14 et 18 mois. La deuxième expérience, décrite dans le chapitre 6, a servi à tester l'influence de quatre complexités de surface différentes et quatre tailles de trous différentes sur la densité, survie et croissance de recrues coralliennes après 9 et 21 mois de conditionnement en milieu naturel. Pour chacune des deux expériences, l'influence du matériau et de la texture sur la colonisation benthique a été testée, ainsi que les corrélations entre communautés benthiques et densité et survie corallienne.

Des structures portant des plaques de recrutement faisant chacune 10 sur 10 cm ont été installées sur la pente externe, entre six et 11 m de profondeur, en 2019. Les plaques ont été régulièrement ramenées en laboratoire, pour pouvoir compter les recrues coralliennes et leur nombre de polype, puis identifier toutes les communautés benthiques. Celles-ci comportaient des macro-algues comme les *Lobophora*, *Dictyota*, algues rouges encroûtantes, algues corallines, turf algal, des éponges, bryozoaires, tuniciers solitaires ou coloniaux, foraminifères coloniaux, polychètes et gastéropodes encroûtants et bivalves. Lors du premier réplica temporel, en 2020, les plaques ont été blanchies à la javel pour identifier les familles de recrues sur base de caractères squelettiques. Les plaques ont ensuite été entièrement lavées pour réaliser un réplica parfait durant la même saison l'année suivante. Les plaques analysées ont ensuite été reposées au même endroit pour suivre la survie et croissance des premières recrues, l'évolution des communautés benthiques, et les arrivées successives de nouvelles vagues de recrues.

Après six mois en 2021, la densité était de 11.2 recrues par 100 cm² sur des faces verticales abritées de la prédation (chapitre 7). Le béton Portland et le béton façonné par imprimante 3D avaient le plus de succès, alors que le verre, la mousse de céramique, le béton poreux, le PLA avec fibres de lins et l'Aquaroche avaient le moins de succès. Les matériaux friables présentaient le plus faible taux de survie, tout comme le béton poreux qui présentait le plus d'éponges, qui peuvent rapidement recouvrir des petites recrues. La fibre de verre et le béton 3D montraient des bons taux de survie, le succès des matériaux variait avec le temps et les recrues survivantes attiraient de nouvelles recrues, soulignant l'importance de faire des suivis à long terme pour se rendre compte de la durabilité et des successions benthiques sur les matériaux artificiels.

Sur des plaques horizontales exposées à la prédation (chapitre 6), il y avait des interactions entre l'orientation, le matériau et la texture de la plaque. En effet, les plaques lisses ne présentaient aucune recrue sur les faces supérieures plus exposées, alors que les faces lisses inférieures avaient autant de succès que les faces complexes. Exposées à la prédation, les pores du béton poreux de l'ordre du mm et les trous carrés imprimés de l'ordre du cm présentaient le même succès. En revanche, la survie était fortement favorisée sur des surfaces lisses, donc sans pores. Ceci est probablement dû à la colonisation des faces poreuses par de

nombreux organismes compétiteurs des coraux, comme les bryozoaires, éponges, *Lobophora,* ou algues rouges encroûtantes. Les trous dans des surfaces très lisses, faisant entre 5 et 20 mm de large et profondeur, sont donc capables de favoriser la survie et la croissance de recrues coralliennes, jusqu'à ce qu'elles atteignent une taille où elles sont moins vulnérables à la prédation. En combinant les résultats des trois derniers chapitres, nous pouvons donner des recommandations précises à des ingénieurs quant à la conception de récifs artificiels adaptés à la survie des recrues coralliennes sur le long terme.

Curieusement, pour les deux expériences, le recrutement observé en 2020 était presque trois fois plus faible que le recrutement sur exactement les mêmes plaques en 2021. Ceci est très probablement lié à l'épisode de blanchissement massif observé à Mo'orea en 2019, qui a tué près de la moitié des *Pocillopora* et *Acropora* de la pente externe et fortement limité les capacités reproductives des colonies survivantes. Il est donc encourageant d'observer une telle augmentation du taux de recrutement moins de deux ans après une perturbation intense. Cela montre à quel point les coraux de Mo'orea font preuve de résilience, en particulier les *Pocillopora* qui sont en général les premiers à recoloniser un site dégradé. Malheureusement, si les perturbations deviennent de plus en plus fréquentes, comme ce qui est prévu par le GIEC avec les prévisions d'émissions de gaz à effet de serre, les coraux n'auront pas le temps de rétablir des populations saines entre chaque perturbation et les récifs risquent de se dégrader durablement.

Bien sûr, malgré des résultats prometteurs, les techniques de restauration corallienne ne doivent pas être considérées comme des solutions miracles. Si les pressions anthropiques locales sont gérées de façon appropriée, la restauration par transplantation ou installation de récifs artificiels peuvent significativement augmenter le recouvrement corallien. Quand ils atteignent la maturité, les coraux restaurés sont des sources importantes de larves, pouvant accélérer le rétablissement de populations saines de coraux. Néanmoins, la réduction de nos émissions de gaz à effet de serre devrait être au centre de nos priorité si nous voulons continuer de profiter des services apportés par les récifs coralliens.

Pour avoir un effet bénéfique, la restauration corallienne doit tendre vers la combinaison de différentes techniques et se concentrer sur les projets les plus durables sur le long terme et applicables à grande échelle. Avec des connaissances grandissantes sur la croissance, la physiologie et la reproduction de coraux au sein des projets de restauration, les résultats de la restauration peuvent être optimisés. Au vu de l'intérêt grandissant du public ou des investisseurs privés pour la restauration corallienne, il est crucial de fournir des recommandations claires et réfléchies sur les différentes étapes de la restauration. Les six chapitres détaillés de cette thèse pourront contribuer à l'optimisation du succès de la restauration corallienne et la combinaison efficace de différentes techniques.

## RÉSUMÉ

Alors que la restauration corallienne suscite un intérêt grandissant, cette thèse fournit un aperçu des méthodes appliquées à Mo'orea, en Polynésie Française, pour améliorer la conservation de la biodiversité corallienne. Premièrement, la croissance en pépinière a pu être optimisée en sélectionnant certaines espèces, leur taille mais également le site de la pépinière. Un changement en profondeur, luminosité et température en passant du lagon à la pente externe a engendré une acclimatation photo-physiologique et une croissance accélérée pour 40% des espèces. Deuxièmement, des *Acropora cytherea* élevés en pépinières ont été transplantés dans trois sites du lagon présentant des paramètres distincts, mais qui n'ont pas influencé leur survie, croissance totale ou maturité. C'était plutôt la taille qui influençait le plus la survie et la maturité. La croissance était initialement favorisée dans le site de référence, mais est ensuite devenue similaire dans tous les sites et dans la pépinière. En troisième, la capacité reproductive des coraux élevés en pépinière pendant plus de 2 ans a prouvé être la même que celle de coraux sauvages. Les taux de fécondation à température ambiante et élevée ont pu être optimisés en sélectionnant certains génotypes parentaux. Les taux de recrutement en lagon ont également pu être grandement améliorés en ensemençant des zones mortes de larves à l'aide de filets, mais les taux de survie des recrues restaient faibles. Ainsi, nous avons testé l'efficacité de huit matériaux artificiels innovants et différents niveaux de complexité en tant que substrats de recrutement. Les résultats montrent que les trous de l'ordre du cm augmentent la densité et la survie des recrues sur des faces exposées, et que la fibre de verre, le béton façonné par imprimante 3D ou le PLA avec fibres de lin présentaient plus de succès que d'autres matériaux. Aussi, une forte densité initiale n'était pas associée à une meilleure survie sur le long terme, soulignant l'importance de tenir compte de la compétition benthique lors de la conception de substrat de recrutement. Tous ces résultats pourront contribuer à affiner et optimiser le succès de différentes techniques de restauration.



### MOTS-CLÉS

Restauration corallienne • Pépinières • *Acropora* sp. • Transplantation • Reproduction • Récifs artificiels



## ABSTRACT

As coral restoration techniques become increasingly diversified, the present PhD thesis provides an overview of methods trialed in Mo'orea, French Polynesia, to enhance the conservation of coral reef biodiversity. First, the growth of corals within suspended nurseries could be optimized by selecting certain species, the initial size of fragments but also the nursery site. A change in depth, luminosity, and temperature from lagoon to fore reef induced an acclimation in photophysiology and a faster growth for 40% of species. Second, nursery-reared *Acropora cytherea* colonies were outplanted to several lagoon sites with distinct degrees of degradation, which did not impact their survival, overall growth or maturity. Instead, their size influenced survival and maturity. Growth was initially increased in the reference site, but soon became similar to that in other sites and to the nursery. Third, the reproductive output of 2-year nursery-reared staghorn corals proved to be the same to that of wild corals of the same species. The fertilization rates at ambient and elevated temperatures could be further improved by selectively crossing certain parental lineages. We also demonstrated that the settlement rates of *A. cytherea* could be enhanced in the lagoon with the use of nets filled with laboratory-reared larvae. While this could prove to be more cost-efficient than transplanting coral fragments individually, the long-term survival of recruits remained extremely low on natural substrate. Thus, eight innovative artificial materials and different levels of complexity were tested for their efficiency as settlement substrates. Our results highlighted that cm-scale crevices increased settlement and survival on exposed materials, and that fiberglass, 3D-printed concrete, or PLA with flax fibers had more success than other tested materials. Also, a high initial settlement was not associated with an improved survival rate during the post-settlement phase, underlining the importance to account for benthic competition when designing coral settlement substrates. These results could help finetune restoration practices and optimize their success by combining multiple techniques together.



### KEYWORDS

Coral restoration • Nurseries • *Acropora* sp. • Transplantation • Coral reproduction • Artificial reefs