FUNCTIONAL RESTORATION AND GROWTH STUDIES

PROJECT PROPOSAL







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Functional Restoration And Growth Studies (FRAGS)

1. Introduction

Coral reefs have been declining at an unprecedented rate for the past few decades^{1,2} with global climate change the driving factor behind most large-scale coral loss events³. A lack of natural recruitment and insufficient time for recovery between disturbance events conspire to make natural recovery unlikely, or impossible in many locations^{4,5}. With over one third of all Scleractinia corals now at risk of extinction⁶ and approximately one third of coral reefs lost from the Philippines since 2009⁷, the continued decline of the worlds coral reefs environments is evident. It is estimated that between 70-90% of coral reefs will be lost by mid-century unless drastic changes take place⁸.

Passive restoration methods (MPA's, no take zones etc.) have been a staple of coral reef conservation for most of the last century, however recent research has shown that optimal conservation outcomes should include both habitat protection and active restoration⁹. In 2019 the 4th UN environmental assembly passed a resolution specific to the management of coral reefs (Resolution 4/13) recognising the role of restoration to achieve biodiversity goals. For corals reefs, enhancing coral cover and abundance via restoration initiatives can ensure sufficient breeding corals remain present on the reef to aid in coral resilience and post-disturbance recovery, whilst 'buying time' for corals as meaningful actions on climate change occur.

1.1 Coral restoration techniques

Active coral restoration methods can be broken down into two main categories, focussing either on the sexual or asexual properties of corals. Corals are continually reproducing and growing via asexual reproduction; it is this trait that coral restoration projects involve 'coral gardening', where coral fragments are grown and nurtured in an intermediatory nursery stage before being moved to their final outplanting spot on the reef proper. Direct transplantation (DT), where corals are transplanted directly onto the reef without an intermediatory nursery stage, makes up 20% of active coral transplantation records¹⁰. This usually takes place when there is an abundance of corals that need to be salvaged due to planned human disturbances, with 20% of all studies using direct transplant showing survival rates at over 90% at end of study¹⁰.

In many cases of coral restoration, healthy corals are "fragged" where parts of the live skeleton are broken off a living donor colony, then used for the re-fragmentation process, however this is detrimental to the donor colony¹¹. "Corals of opportunity" (COPs) solve this problem of causing damage to the donor colony; COPs are corals that have previously been broken from a parent colony, either through natural or human mechanical processes. As these corals are not stabilised or attached to a hard substate, the likelihood of them growing into a new full, colony is small; if they are manually attached to a hard surface, the likelihood of survival into an adult colony greatly increases¹². The average survival rate (at end of study) for COPs across 75 case studies was 89.5% whereas the average survival rate for transplanted fragments across 142 case studies was 75.6%¹⁰. As it is not stated in this review of studies if the COPs were fragmented once collected, or the size of any fragments/COPs used, it is hard to directly compare these results; however, this data does suggest that using COPs can yield high transplant survivorship.

Using COPs naturally favours branching morphologies as they are the most likely to undergo natural dissociation from the parent colony. Branching morphologies are usually favoured for use in coral restoration; they tend to be the fastest growing, offer the most in terms of structural complexity and they are the most suited for propagation via fragmentation¹³. If the goals of restoration are to include resilience to existing or future stresses, the consideration of genetic diversity is crucial¹⁴.

Most nursery structures are constructed between five and 15 meters deep (dependant on the primary coral species/genera targeted), and preferably at a similar depth to where donor colonies or the fragments were retrieved¹⁵; any shallower and they are at risk from mechanical dislodgement via wave action, any deeper and they will suffer from reduced growth rates due to light attenuation. This shallow depth range also offers increased dive time for routine maintenance and care of the fragments¹⁵. Regular cleaning/maintenance is often required to mitigate the effect of algae growth and possible smothering of the fragments. Placing nursery sites close to a natural reef can reduce this required cleaning/maintenance effort, as local fish and invertebrate grazer populations will assist in algae removal. The benefits of herbivorous fish outweigh the costs of corallivory in coral nurseries close to living reef¹⁶, and also the presence of local invertivorous fish populations can reduce the damage caused by invertebrate coral predators¹⁷.

Although using the asexual properties of coral for active restoration is currently by far the most widelyused technique, it does have drawbacks. It can only be used on relatively small scale¹⁸, it has a high resource use in terms of time and monetary budget, and using asexual reproduction techniques reduces genetic diversity and therefore potentially reduces resilience from future diseases and stressors¹⁹.

	% Survivorship	SEM	Number of studies			
Genus						
Porites	76	2.6	18			
Montipora	73	3.7	10			
Pavona	72	7.3	6			
Acropora	69	2.3	18			
Pocillopora	68	4.2	10			
Stylophora	67	5.6	2			
Seriatopora	67	5.6	2			
Echinopora	57	7	7			
Merulina	52	8.9	4			
Morphology						
Massive 73		4.5	12			
Branching	71	1.4	30			
Foliose	62	5.5	12			
Encrusting	58	7.6	5			

Table 1: Mean survivorship of coral fragments at the end of the survey period throughout the
Philippines; adapted from Boström-Einarsson et al,. 2020 ¹⁰

To date, approximately 9% of active restoration projects have used the sexual properties of corals¹⁰. Direct coral seeding involves collecting coral gametes from spawning corals and then allowing them to develop in a protected area before being directly applied onto the reef. With the potential to drastically improve the number of coral recruits reaching adulthood on a reef, trials have been ongoing for over ten years now²⁰, but success varies hence it is yet unknown if this is a viable technique for large scale reef restoration. A 2015 study in Palau²¹ showed no significant difference in coral recruit density between seeded and control plots 13 months after seeding with over one million *Acropora digitifera* larvae (although high background recruitment in the control site may have affected the results of this study). However, a 2017 study²² showed a significant difference between seeded and non-seeded plots after a three-year period. As such, this technique may become a valued tool for active reef restoration where larval supply and hence recruitment success are a limited.

A lack of hard substrate for coral attachment is one of the most common non-climate related barriers to natural recovery of coral reefs²³, which will be unchanged by the implementation of coral seeding.

Responding to this, A 2017 study²⁴ used "seeded" concrete tetrapods and after approximately one year, 56% of units harboured at least one *Favia fragum*; this seeding technique eliminates the limitations caused by the lack of suitable settlement substrate. It is suggested that reef rehabilitation methods that aim to harness coral sexual reproduction might better focus on rearing juveniles through early post-settlement mortality bottlenecks²².

Another option for boosting coral recruitment in the presence of detrimental environmental conditions (a lack of suitable hard substrate attachment points) is seafloor stabilisation and the construction of artificial reefs (AR) to enhance larval settlement ²⁵. One of the most successful uses of ARs is as a nursery habitat for coral transplants²⁶; as long as the mortality of initial transplanted colonies is low, they will soon add to and increase the local coral larval production²⁷. ARs have been shown to have a high (71%) success rate where their main objective for placement was to increase hard coral cover²⁶. It is not just the local coral population that benefit from the presence of ARs; a meta-analysis of 39 studies by Paxton et al.²⁸ showed no difference in fish community metrics between natural and artificial reefs.

Both the materials used and the complexity of the artificial reef structures will influence the success of coral attachment and the development of the subsequent benthic community^{29,30}. It is important to be wary of the ecological footprint of the material used; cement is a commonly used construction material in AR's, however production of cement is responsible for 5-7% of global carbon emissions³¹.

1.2 Artificial Reef Case Study: Dauin (Negros Oriental, Philippines)

Dauin (Negros Oriental, Philippines) presents the optimal case study to determine the reseeding potential of artificial reefs in recruitment poor locations. Three artificial reef sites can be found along the Dauin coastline, comprised of varying structure types, materials and complexities. Due to the recent super typhoon, whole colonies have been uprooted from their base and are undergoing breakage, scouring and burial due to their inability to reattach to the unstable reef substrata. This project will utilize these susceptible corals (Corals of Opportunity; COPs) by reattaching their fragments to various artificial structures. Using artificial structures to transplant COPs (rather than suitable natural substrate) ensures space availability for incoming larvae, which is essential for maintaining site resilience.

The Lipayo artificial reef site in Dauin (Fig. 1) has been selected for this study. The various artificial structures were deployed between 2007 and 2016, and contain low to no coral cover as this artificial reef is in a recruitment limited location. Structures with very low coral cover include the large cages, and the bar pyramids (Table 2). However, there are a few structures that have high coral cover; both the cement pyramids and tyre clusters have so much coral cover that has sprawled out from the structures that it appears as natural reef, until the structures beneath are seen. A comparison of hard coral cover on two structure types at Lipayo Artificial Reef between 2020 and 2022 shows coral cover on the shallow cages (6m) approximately doubled from 7.4 to 14% during the study period (Fig. 2). This is far below the mean hard coral cover throughout Filipino reefs (22.8%⁷), and additionally shows there is ample space to be used as attachment points in coral restoration projects. The pyramids at approximately 18m show minimal change in hard coral, with a decrease from 1.5 to 0.6% cover (Fig. 1), whereas hydroids and algae show dramatic increases (7.9% and 11.6% respectively).

Through a series of detailed monitoring and research efforts, the Institute for Marine Research will determine the ability for previously coral-devoid ARs to become preliminary refuges to COPs, and later result in the sexual maturity of these COPs with the potential to reseed nearby and previously disturbed natural reefs. This will be the first project to define the reseeding potential of COPs grown onto artificial structures. By utilizing artificial structures devoid of coral due to limitations in

background natural recruitment, yet are proven to be withstanding against incoming typhoons, could drastically improve post-disturbance survival of fragmented colonies.

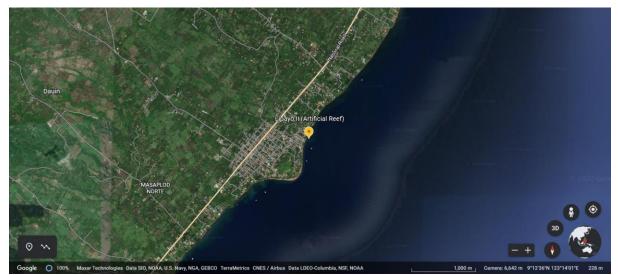


Fig. 1: Satellite map of study location (GPS coordinates 9.192531,123.272175).

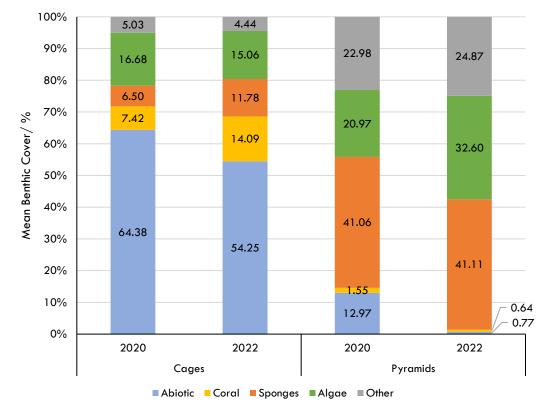


Fig. 2: Comparison of the benthic cover (%) at the Lipayo cages at 6m (left) and deep pyramids at 18m (right) between 2020 and 2022.

Table 2: Structure types at Lipayo Artificial reef, the number of each type, material, size, depth range, distance apart and benthic composition (images of structure types on pages 9-11). Greyed out rows indicate structure types not suitable for the FRAGS project. See Fig. 4 for images of structure types.

Structure	No.	Material and dimensions	Depth range	Distance apart	Colonisation
Bells (long and short)	~140	Cement. 2.3m circumference, 0.6-0.8m tall	5.5-19m	Cm's/touching at base – metres apart	Complex benthos, variable coral settlement
Cake stands	20	Cement. ~1.4m tall, 0.9m wide (middle shelf)	3 in shallow area (6-7m), 17 scattered throughout deeper water (12-18m)	5 - ~20m apart (many situated in close proximity to bells)	Complex benthos, including coral settlement and growth
Pyramids	16	Rebar. ~1.5m tall (along one edge) by 1m wide (distance between legs at base)	One shallow (6.5m), all others scattered from 13- 15m, in two distinct areas (see site map)	2 - 10's metres	Little coral settlement, mostly algae and sponge
Pyramids (deep)	4	Metal/rebar. Varying sizes (largest is 2.2m tall along one edge by 1.5m at the base)	17-18m	One cluster, maximum few m's apart	Heavily colonised (0.8% bare substrate (abiotic)) and complex benthos with some coral (0.6%), high algae (33%) and sponge cover (41%) (Fig. 2)
Cages (small)	2	Rebar. 2.1x1.2x0.6m (longer cage)	5.7-6.1m	3m (corner to corner)	Coral settlement and growth appears highest on these structures (14%), some algae (15%) and sponge (11%) growth also present (Fig. 2)
Cages (large)	2	Rebar. Shorter cage is 4.2x3x2.1m, longer is 12.5x3x2.1m.	16-18m (shorter cage) and 11.5-14.3m (longer cage)	~10m apart (corner to corner)	Very little coral cover, mostly hydroids, sponge and algae
Pyramids	2	Cement. Size of structure unclear	12-13m	3-4m	Structures so heavily colonised it is difficult to see where structure ends and 'natural reef' begins to sprawl. High coral cover, but other benthos present. Not suitable structures for FRAGS (too high natural coral cover).
Tyres	3 sets	Metal/rubber. Diameter max 0.5m per tyre	9.7-14m. In distinct clusters, some have ~6, others individual.	Clusters are 10's m away from each other.	Heavily colonised by corals, as well as other benthos. Not suitable structures for FRAGS (too high natural coral cover).

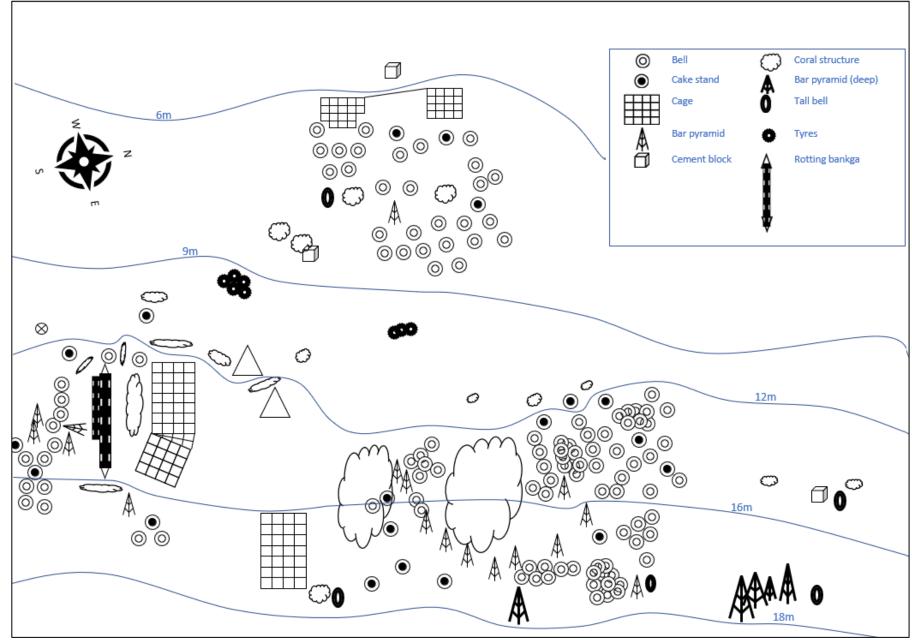


Fig. 3: Map of Lipayo Artificial reef, including various structure types.

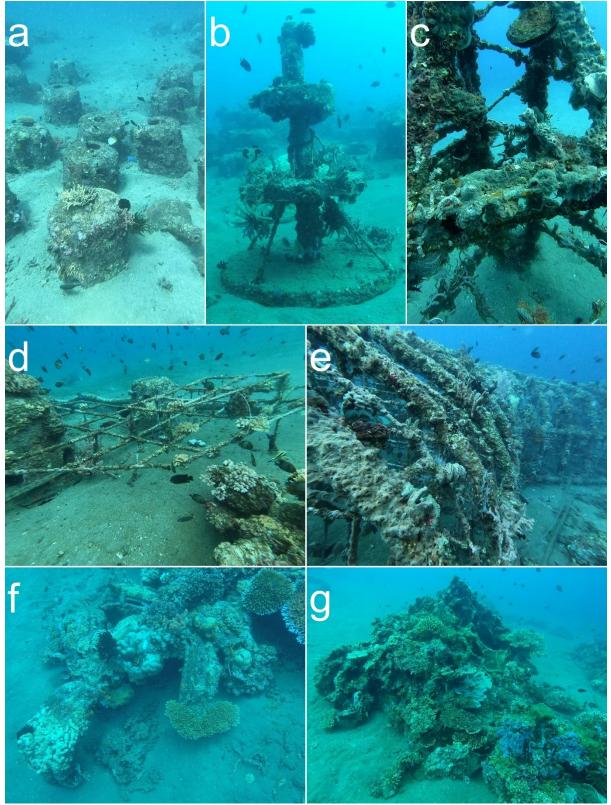


Fig. 4: Various structure types at the Lipayo Artificial Reef, Dauin (a) bells, (b) cake stands, (c) bar pyramids (deep), (d) small cage, (e) large cage, (f) tyres, (g) cement pyramids.

2. FRAGS Research Questions

- 1. What factors promote the post-settlement survival of COPs onto artificial structures?
 - a. Depth
 - b. Material of artificial structure (metal and concrete)
 - c. Pre-existing benthos (competition)
 - d. Microhabitat (structure and orientation see Fig. 5 below)
 - e. Distance travelled for COPs from collection to outplanting location
- 2. How do the following COPs life history traits affect survivorship and growth rates?
 - a. Coral morphology
 - b. Coral species (and baseline growth rates)
 - c. Reproductive strategy (brooding or broadcast)
 - *d. Size of fragment (50/75/100 mm ±5 mm)*
 - e. Fragment type and orientation
 - f. Condition of COPs when found (e.g. buried in sand, bleached, scoured)
- 3. How do the following ecological variables affect COPs survivorship and growth rates?
 - a. Turf algae
 - b. Predation (herbivory/corallivory)
- 4. How does the planting of fragments onto artificial reef structures affect the reef fish community (species diversity, abundance, size structuring, functional groups) and over what time period do these changes to occur?
- 5. Can the outplanting of COPs facilitate larval settlement due to changing chemical cues and 3-Dimensional structure?
- 6. How long after out-planting fragments is sexual maturity reached, and do any of the above variables affect this?

a. What are the natural recruitment levels at this artificial reef site? How might coral larval retention, settlement and survivorship change as a result of outplanting?

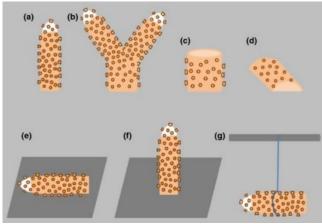


Fig. 5: Type of branched coral fragments and orientation of fragments. (a) Single branch tip. (b) Dichotomous branch. (c) Cylindrical middle section of branch. (d) Flat fragment. (e) Horizontal orientation. (f) Vertical (upright) orientation. (g) Suspended fragment. (Leal et al., 2016³²)

3. Methodology

3.1 Corals of Opportunity (COPs)

All corals obtained for FRAGS will be Corals of Opportunity (COPs), i.e. pieces of live coral that have previously been broken off the parent colony (through natural or anthropogenic causes) and are currently undergoing scouring/burial due to their inability to reattach to unstable reef substrata.

COPs will be collected at a variety of locations across the Dauin inshore reef. COPs will be identified to species level (where possible), and tagged with a Donor ID which comprises of the following information:

- Location, date, time and depth of collection
- Condition of COP (healthy, bleached, diseased etc.)
- Genus/species
- Size
- Number of fragments produced from COP

3.2 Fragmenting

Fragmenting will be done with care to limit damage to polyps surrounding the break. To break the COPs into appropriate fragment sizes, we will use pliers for branching corals (or PVC cutters for thicker branches), and a hammer and chisel for massive corals. Fragmenting takes place out of water so care will be taken to minimise the amount of time the fragments are not submerged. The fragments will be placed in a separate container to the donor COPs.

All fragments will be given a unique Frag ID to ensure traceability of all fragments is maintained; from collection (Donor ID) to out-planting (Frag ID). Fragments characteristics that need to be recorded (along with Donor ID) are:

- Length of fragment in mm (50mm/75mm/100mm, ±10mm)
- Type of fragment; single branch tip/dichotomous branch/cylindrical section

3.3 Outplanting

Attachment sites will have been previously identified and thoroughly cleaned, using paint scrapers and wire brushes to expose the substrate. The fragments will be attached to the substrate by gently pressing a grape-sized ball of two-part epoxy resin onto the cleaned attachment site and then pressing the fragment firmly into the resin. The resin will then be smoothed so there are no gaps between the fragment and the epoxy. If the fragment is too large to be attached by a single small epoxy ball, one larger one or multiple balls shall be used at several attachment points across the fragment. Once the fragment is introduced to the epoxy it must not be moved, as any movement would break the bond between the epoxy and the fragment, increasing the chance of subsequent detachment from the structure.

It is essential to ensure fragments are a sufficient distance from each other; 200mm apart is the minimum distance to be used during FRAGS. It is important to follow the pre-determined fragment orientations and homogeneity (all adjacent fragments have same Donor ID) chosen for fragments to ensure experimental design is followed and to reduce stress/competition respectively.

All data to be collected on fragment out-planting:

- Frag ID
- Location and structure ID planted on
- Depth
- Orientation

- Length of exposed fragment (not including the part of fragment that is submerged in resin, so subsequent linear extension can be accurately calculated)

3.4 Monitoring

3.4.1 General monitoring and maintenance

All data to be collected on fragment growth and survival (per excel "3. Monitoring") for **all fragments**:

- Date and time of survey
- Frag ID
- Condition (alive/dead cause of death where visible, e.g. disease, predation)
- Linear extension (branching fragments)
- Ecological volume (all fragments)

General monitoring will be repeated at 2 weeks, 8 weeks, 3 months, 6 months, 9 months, 12 months and 24 months post-planting.

To maintain the health of the newly outplanted fragments, the area surrounding the fragment will be cleaned to remove new algal growth. This cleaning maintenance will take place often (frequency to be determined upon outplanting and subsequent algal growth rates) to ensure fragments are not outcompeted by algae. It is likely this routine maintenance will only be required during the early phase of this project, as larger fragments will be more resilient to algal competition.

3.4.1.1 Coral health

The health of the entire fragment will be assessed and categorised as one of three; alive/ partial death/dead. The nature of the death will be recorded in video footage.

- If disease is found on any of the fragments it will be recorded and the fragment will be epoxy banded to decrease the spread to the rest of the colony. If this does not solve the issue, the diseased portion will be pruned off and discarded far from other healthy colonies that could potentially be affected.
- \circ $\,$ Any coral predators found in the vicinity of the fragments will also be recorded and then removed.

3.4.1.2 Linear extension

For branching colonies, linear growth and total linear extension will be measured from the base of the fragment using callipers (Fig. 6).



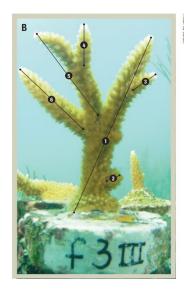


Fig. 6: In-situ measurements of nursery fragments; Image Α shows linear growth measurement (maximum height). the tissue Image В shows extension measurements, all measurements 1- 6 were added together to get total live tissue for the fragment (Johnson et al. 2011^{15}).

3.4.1.3 Ecological Volume (EV)

Measurements of maximum dimensions for length, width and height (Fig. 7) will be collected using callipers, from which ecological volume (EV) will be calculated according to the following equation: $EV = length_{max} \times width_{max} \times height_{max}$

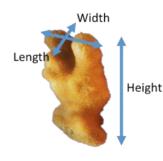


Fig. 7: Ecological Volume (EV) measurement (Chung & Hintzsche,. 2017³³).

3.4.2 Additional monitoring

3.4.2.1 3D Modelling

For **selected fragments**, 3D modelling will take place at 0 weeks, 8 weeks, 6 months, 12 months and 24 months post-planting.

Video footage of individual fragments will be used to extract stills to be used for Structure from Motion (SfM) photogrammetry. Two video passes will be completed per fragment; one with the camera approximately 15cm from the fragment (for overall shape of fragment), and a second pass much closer to the coral (7-10cm) for finer polyp detail. The scale bar will be placed in a consistent and secure location each time the fragment is modelled.

For each pass, the video will start side-on (0° relative to coral fragment) and complete a full loop, rotating in a circle as we slowly move around the focal object (fragment) (Fig. 8). Once the first loop is complete, the camera will be angled approximately 45° above the fragment for another full loop. Lastly, a small loop at 85° (almost top down) will be completed, which should have the entire scale bar in the video. Additional video should be taken of areas where complex branch intersections or overlapping portions of the coral will require increased resolution to accurately build. Stills will then be extracted from video footage to build the 3D model and take 3D metrics in RealityCapture.

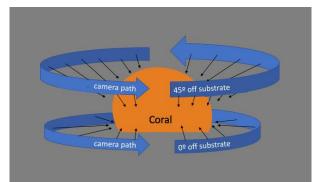


Fig. 8: Optimal camera paths and angles to photograph a single coral colony for 3D photogrammetry (Million and Kenkel 2020³⁴).

3.4.2.2 Fish community surveys

Fish community surveys will be completed every 12 months. When this data is to be collected, it must be the first procedure to be completed; no divers should enter the survey area until this is complete. If divers are seen in the area, the team must wait 5 minutes before starting video collection. From video footage, the following fish community metrics will be examined; species diversity, abundance, size structuring, functional group diversity. It will likely take several years for this data to show significant changes from the fish population at the beginning of the study.

The SVS will be set up at the pre-determined locations (with a wide view of the fragment planting area) to collect stationary video footage for a survey duration of 5 minutes. Footage will be analysed in EventMeasure.

3.4.2.3 Benthic assays

Benthic assays will be completed at 0 weeks, 3 months, 6 months, 12 months and 24 months postplanting. One image will be taken per fragment planting area on the structures, perpendicular to the structure surface. The camera will be held 0.5m from the fragment, using a plumb line to measure distance and to centre the image with the fragment in the centre. The surrounding benthic community on the artificial structure will be analysed using CPCe.

3.4.2.4 Onset of Sexual Reproduction

24 months after planting, surveys on the onset of sexual reproduction will begin. For all coral fragments, gravid colony checks (presence of eggs in colony) will be completed 2 weeks prior to the identified spawning schedule for that species/genus of coral.

Further studies on sexual reproduction of coral fragments will include the deployment of settlement tiles (5x5cm travertine tiles) to investigate coral larval retention, settlement and survivorship at this artificial reef site.

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