Coral reforestation

July, 2020

In this document a procedure is defined that reflects the findings of our conservation work in East Africa and has used the available literature to optimize genetic diversity in our coral farming/reforestation projects. The procedure is written for coral reforestation on natural substrate on the basis of asexual propagation. The procedure will be under continuous development coming years depending on new experiences and research.
The *cursive text* reflects relevant excerpts from literature and the reef restoration course from Reef Resilience Network, NOAA, The Nature Conservancy and Coral Restoration Consortium. Some definitions are given on the last page.

Procedure: collection of COOs, farming & outplanting

a. Collection of Corals of Opportunity

Corals of Opportunity or COOs (see d. definitions) are collected at different locations and brought back to the coral farm. The GPS coordinates of the COO locations are registered. Locations for collecting one species must be at least 50 meters apart, having the best chance of collecting different genotypes. We assume a different location implicates that we will find a different genotype for the concerned species. Collecting several donors of the same species at one location would have the risk of collecting the same genotype. Per species we want at least 15 genotypes in the farm.

To anticipate climate change we want to include heat-resistant genes in the farm. For that reason we have to go to different habitats regarding temperature. We make 4 COO dives at a habitat similar to the habitat where the outplanting is planned, 2 COO dives in a habitat with intermediate temperature and 1 COO dive in a habitat with high temperature. For example, when the outplanting is mostly done at 8-10mtr depth, you could aim for 4 COO dives at a similar reef at this depth, 2 COO dives at 5-7mtr depth and 1 COO dive at 1-5mtr depth.

We focus on the corals we want to grow ie a reflection of the reef we want to restore. Possibly some species have disappeared on the concerned reef and we can consider to bring them back if it has a success probability.

While collecting the COOs we take into account the required amount of frags (see b. Coral farming) we need. In the farm we want to grow 20 frags per genotype so a donor genet should give 20 frags. A few more to compensate farm mortality is ok but the total should stay under 25 frags. We limit the cloning to a maximum per donor genet to obtain a high genetic diversity. For example, for a COO a 20cm piece of branching coral is enough. If a COO is square, a 10x10cm piece is enough, also depending on the size of the corallites.

The coral divers have to register the GPS data of the COO location. Planning for next COO dives involves location planning. Coming back to one COO location to find other species than we found before can be part of the planning. But obviously not of the species that were found earlier, only maybe in a far future assuming the coral gene mix changes over time on that reef.

Coral collections should capture as much genetic diversity as possible. In a best-case scenario, the genotypes of corals brought into nurseries would be known. If genotyping is not possible, fragments should be taken from physically separated reefs or populations to increase the chances of obtaining unique genotypes (Johnson et al. 2012).
"Thickets" of branching corals often have high numbers of clones and should be avoided during collections.
Collect coral fragments from as many physically separated reef areas as possible to increase the likelihood of obtaining unique genotypes

b. Coral farming

Our nursery consists of steel coral tables covered with a plastic mesh on top. This mesh is also used in the aquaculture business. Tables are approx. 250x70x70cm. On the legs of the tables we put a part of a recycled water container or similar. This part is curved downwards to prevent Crown of Thorns starfish to climb up.

We 'frag' (i.e. cut in fragments) the donor genets with plyers and the tougher boulder corals with a hammer and chisel. The frags are glued on cement plugs i.e. a flat disc with a pin made entirely of cement. The cement plugs are made in the sand that is used as a mold in which the cement is poured. It is recommended to make a wooden plug that can be used to prepare the molds in the sand. The wood has a smooth surface so sand will hardly stick to it.
After about 4 days of drying it is recommended to keep the plugs in the ocean for 2 weeks before use.

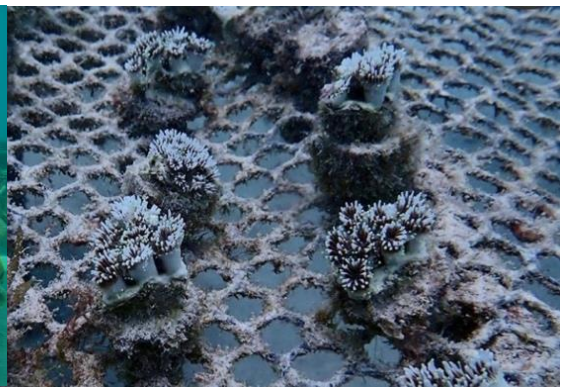
The glue we use to attach the frag to the cement plug is special underwater glue used in aquaria called Reef Construct from www.aqua-medic.de. We have seen other projects using superglue but in order to apply that the plug and coral have to be taken out of the water and surfaces have to be dry. With Reef Construct we don't have that problem. For our current projects Aqua Medic in Germany sponsors us by supplying Reef Construct free of charge.



The coral tables are divided in 10 segments. The amount of required tables can be calculated by #genotypes p. species X #species / 10. For example, you want 15 genotypes per species and you concluded that a reflection of your reef is 20 species, 15 x 20 / 10 = 30 tables. It is possible with tables of 250cm to have 12 segments, in this example this results in the need for 25 tables.

Tables are labeled with letters A, B, C, etc. On a table 10 clearly separated segments are labeled with number A1, A2, A3,.. resp. B1, B2, B3,... called 'table-segments'. Each table-segment receives 20 frags of one species from one donor coral, or a few more to compensate for mortality.
So for example the table-segment B2 has 20 genets or 'juvenile corals' of 1 genotype (of 1 species). We assume that this genotype is unique and you will not find it elsewhere in the farm. Without DNA sampling you will never know 100% of course but the probability factor is high if you work this way.





Coral farm in Vipingo Coral farm on Zanzibar

Coming back from a COO dive the empty segments are filled with the COOs and following info is registered on a tablet underwater or afterwards on the basis of photos: COO location, species, table-segment.

Then the big work starts i.e. cleaning of the juvenile corals and coral tables, making sure the corals grow undisturbedly. As in nature, there is a lot of competition for space. You will see this happening on the tables. Algae, sponges and plants start growing on the table and eventually on the coral plug next to the coral. That is the material we want to remove with care before it overgrows or suffocates the juvenile coral. A simple toothbrush will do but to work faster a domestic brush is more convenient. Take care with cleaning especially when the frags are recently glued to the disc since the glue might not be strong enough to withstand uncontrolled movements. After the juvenile coral grows it will fixate better on the plug.

Depending the species and the environment, the juvenile corals may take 4-12 months to grow sufficiently. The bigger the juvenile coral colony, the higher the success rate i.e. the lower its mortality rate in nature. Branching corals often grow faster and attach quicker on the cement plugs than e.g. boulder corals

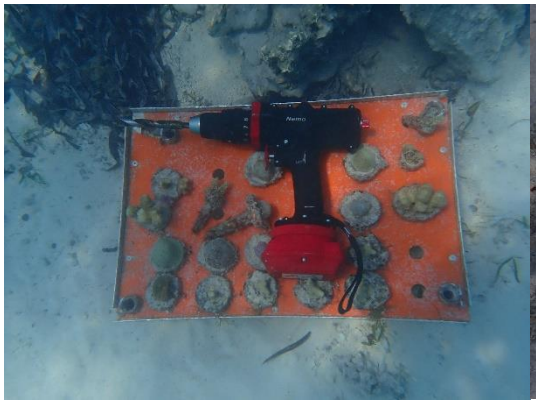
Genotypes of corals in the nursery should be tracked so genetic diversity can be maintained and genotypes can be fragmented separately. This can be done by placing different genotypes on separate nursery structures and maintaining a detailed map of the nursery. Labeling can also help nursery personnel avoid mixing up genotypes.
In general, nurseries should target min. 15 genotypes per species for rearing and outplanting. Coral restoration strategies using 10–35 randomly selected local donor colonies will retain at least 50–90% of the genetic diversity of the original population.
Coral-List Digest: 'When we identify particularly resilient genotypes, how can we rationally discard asexual propagation (fragging) of these genotypes as a tool for active reef restoration? If we can use asexual propagation methods to outplant resilient coral fragments that have already reached the size refuge that drastically increases survivorship, we should.' Jonathan Barton.
If an outbreak occurs, diseases are often managed by placing diseased corals in a quarantine area, removing diseased corals entirely or utilizing an epoxy ring over the disease margin (ps touch with gloves).

c. Outplanting

Dedicate areas for outplanting (=transplanting) and make a comprehensive plan before you start so everybody knows what, why and where to do it. Ideally, first talk to especially net fishermen to (temporarily) protect an Outplant Area (= OA).
OAs have to be at least 50mtr apart meaning the boundaries of the OAs are 50mtr apart.


If we aim for 50% genetic diversity in an OA we should outplant 15-20 genotypes per species. So we plant one genet (=juvenile coral) per genotype and at least 15 different genotypes per species. The juvenile corals are placed near each other at always a distance of about 50cm. Far enough to grow without direct competition and close enough to sexually reproduce once they mature. Optionally, divide this amount in 2 groups of closely positioned genotypes.

At the first visit to an OA we outplant all the genotypes per species that 'matured' in the farm and register them. We have to know exactly which OAs received which species/genotypes. At a 2nd visit to the concerned OA we first check what has been outplanted already to prevent duplicates and bring the next batch of matured juvenile corals.

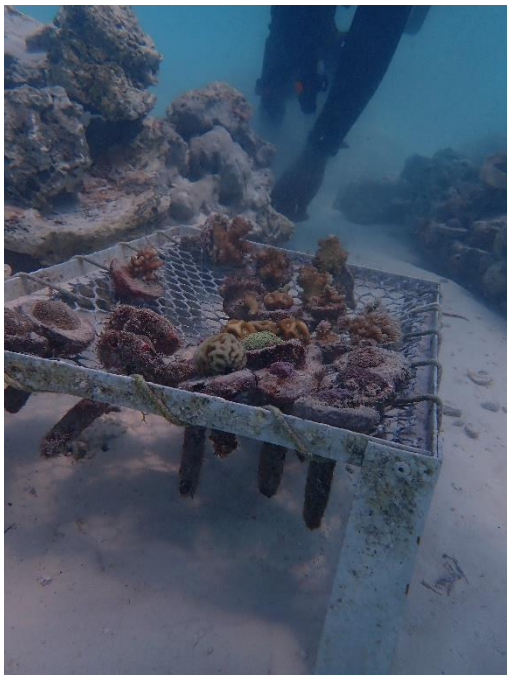


We select OAs where the coral cover on the substrate has been 40-100% in the past and is reduced to 10-20%. If coral cover is 0%, chances are that nothing at all will grow on the concerned area; if coral cover is more than 20% it is possible the area is effectively recovering already naturally. The 10-20% threshold is empirical, we have no scientific data on this.

We make holes in the substrate with the use of an underwater Hammer diver drill (Nemo). In a hole, we put a little cement mixture inserted through a piping bag (as used in the kitchen). With plyers we customize the length of the pin of the plug to make sure the pin will be

entirely inserted in the hole. In a rotating movement we manually insert the pin of the plug in the hole. It is important that the pin is completely inserted so the disc lies flat on the substrate. This makes the construction more robust and less vulnerable for wave action or predator impact. Also, this way we achieve alignment of the substrate area with the disc area so the juvenile coral can grow over the disc directly towards and over the substrate.

If we want to increase genetic diversity in the future we can come back to the concerned OA with next batches of different genotypes when they are ready. For example, 90% genetic diversity means more than 35 genotypes per species per OA.



Outplanting branching corals to be done at size 5-15cm, massive 4-5cm.
Outplanting a mixture of coral genotypes is critical for ensuring cross-fertilization of corals within a reef site. Outplanting several genotypes in close proximity will increase the chances of successful sexual reproduction, helping enhance site-wide genetic diversity and coral population recovery. It is recommended to outplant at least 10 genotypes per coral species in a site with at least 3 replicate coral colonies (NOAA 2016).

d. Definitions

Genet (plural **genets**): a group of genetically identical individuals (coral colonies) that have grown in a given location, all originating from asexual reproduction of a single ancestor; a group of ramets.
Genotype: the genetic constitution of an organism. The genotype determines the hereditary potentials and limitations of an individual from embryonic formation through adulthood. Among organisms that reproduce sexually, an individual's genotype comprises the entire complex of genes inherited from both parents.
Corals of Opportunity: coral colonies dislodged from the reef from unidentified causes such as bioerosion, storms, or anchor damage.