Summary of replenished coral reef sites in SWCMR and TAMR 2017-2020

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Executive Summary

This report on replenished coral reef sites in South Water Caye and Turfeffe Atoll Marine Reserve is the final technical report required under the MCCAP sub-project entitled "Repopulate reefs within replenishment zones of Turneffe Atoll Marine Reserve (TAMR) and South Water Caye Marine Reserve (SWCMR) with temperature resilient coral varieties" (Contract Number: MCCAP/SER/05 and ID number: P131408-BZ/C-2). The objective of this consultancy was to support the implementation and expansion of propagation and restoration practices in TAMR and SWCMR in Belize. The methods and work plan were outlined in the approved Inception Report with some exceptions described here; the single trial with hemp versus polypropylene rope in a SWCMR nursery, and the adoption and adaptation of micro-fragmenting corals for direct outplanting (by-passing nursery time) in both MPAs. One of the newest revelations from this work was the marked difference in growth rates (*A. cervicornis* in nurseries), north to south, with the northern corals/sites growing significantly slower than southern corals/sites, which delayed outplanting this species in TAMR. Almost 29,000 fragments were outplanted within both MPAs (18,238 fragments in SWCMR and 10,689 fragments in TAMR), far exceeding the anticipated minimum of 6,000 fragments per MPA. Four permanent, measured plots were created in each MPA, totaling 648m^2 in SWCMR and 733m^2 in TAMR. Once these plots were outplanted, no additional corals were added so that the annual photo-mosaics may track natural recovery/growth of replenished sites. Only 3,861 of the 18,238 fragments outplanted in SWCMR are inside these measured plots, so in fact there are eight outplant sites at SWCMR. Similarly in TAMR, only 3,195 of the 10,689 fragments outplanted there are inside the four measured plots and there are least four additional outplant sites not measured. While photo-mosaics were conducted on all four plots in both MPAs prior to outplanting in 2018, and again in 2019 and 2020, none of these has yet been processed or annotated. Shared here are time series photographs of multiple coral taxa, in multiple shallow reef habitats, highlighting various outplanting methods. Nurseries were installed 2016-2018 and so all outplanting began in 2018 with one notable exception: the first directly outplanted *Acropora palmata* micro fragments were placed on the SWC shallow forereef in April 2017 and now have over three years on the reef, surviving through two major bleaching events (2017 and 2019). This report describes the results of the outplanting process 2017-2020, lessons learned, and recommendations for future reef replenishment work.

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Introduction/Background/Project Justification

Climate change is believed by the majority of marine scientists to be the most serious threat to corals and their ecosystems today (Aronson and Precht 2006; Baird et al. 2009; Hoegh-Guldberg and Bruno 2010; Lesser 2011), with global warming causing increased severity and frequency of bleaching and coral mortality (Hoegh-Guldberg et al. 2007). Coral reefs are generally recognized as the most vulnerable of the planet's ecosystems to the impacts of climate change (Donner et al. 2005). An estimated 19% of the world's coral reefs have been lost and a further 35% are seriously threatened (Wilkinson and Souter 2008), and one-third of all reef-building corals are considered to be at risk of extinction (Carpenter et al. 2008). Some authors estimate 60% of all live corals could be lost by 2030 and state that current management practices must undergo radical changes to become effective (Hughes et al. 2003).

Widespread coral loss due to thermal stress and mass bleaching has already occurred (Hoegh-Guldberg et al. 2007) and Caribbean reefs are particularly impacted, with lower coral cover presently than at any time in geological history (Greenstein et al. 1998). The Caribbean as a whole has lost an average of 40% of its absolute live coral cover since the late 1970's (Gardner et al. 2003) and most of this is accounted for by the wide-spread loss of two Caribbean acroporids, *Acropora cervicornis* (Lamarck 1816) and *A. palmata* (Lamarck 1816), whose mass mortality is attributed to hurricanes, bleaching and disease (Aronson and Precht 2001; Bruckner 2003). These two species are the fastest growing, main reef building species in the Caribbean, previously dominating both the shallow and intermediate depths; their combined abundance has been reduced by more than 95% Caribbean-wide and they were placed on the IUCN's Red List in 2008 as Critically Endangered, one step away from Extinction in the Wild (Aronson et al. 2008).

In Belize, coral reefs were valued for their ecosystem services (shoreline protection, nursery habitat and aesthetic/tourism value) at over US$370million/year (Cooper et al. 2008). The national average coral cover is currently just 15%, yet both Turneffe Atoll and South Water Caye Marine Reserve are labeled as "poor" with coral cover between 5-9% (Kramer et al. 2015).

The most widely recognized climate change adaptation option for coral reefs is to increase coral reef health through the management of local stresses such as pollution, sedimentation, and overfishing (Buddemeier et al. 2004). But with ongoing work at Laughing Bird Caye National Park (LBCNP) in southern Belize since 2006, an additional option has been explored and now validated: the identification and propagation of bleaching resistant and/or resilient corals, their cultivation into second/third generation fragments, followed by transplantation to reefs where thermal stress has decimated coral cover (Carne 2008, 2011; Bowden-Kerby and Carne 2012). Restoration techniques have recently become more accepted as conservation tools in recognition of such rapid and continued reef degradation (Jaap 2000; Rinkevich 2005; Baums 2008; Baums et al. 2010; Lirman et al. 2010; Johnson et al. 2011; Young et al. 2012; Rinkevich 2014).

Belize, under the leadership of the Ministry of Agriculture, Forestry, Fisheries, the Environment and Sustainable Development with fiduciary management assistance from the Protected Areas Conservation Trust (PACT) as the National Implementing Entity (NIE) and the World Bank as Multilateral Implementing Entity (MIE), is responsible for the implementation of the Marine Conservation and Climate Change Adaptation Project (MCCAP) in the coastal areas of Belize. The Project Implementing Agency Group (PIAG) housed within the Fisheries Department and staffed by full-time and part-time consultants is responsible for the coordinating MCCAP implementation. The PIAG consists of a Project Coordinator (PC), a Senior Technical Officer (STO), Administrative Officer, staff from Fisheries Department, and fiduciary staff of PACT.

MCCAP is a five-year project designed to implement a priority ecosystem-based marine conservation and climate adaptation measures to strengthen the climate resilience of the

Belize Barrier Reef System and its productive marine resources. Specifically, the project will support:

i. Improvement of the reef's protection regime including an expansion and enforcement of the Marine Protected Areas (MPAs) and Replenishment (no-take) Zones in strategically selected locations to strengthen climate resilience,

ii. Promotion of sustainable alternative livelihoods for affected users of the reef, and

iii. Building local capacity and raising awareness regarding the overall health of the reef ecosystem and the climate resilience of coral reefs.

MCCAP will benefit three Marine Protected Areas (MPAs), namely, the Corozal Bay Wildlife Sanctuary (CBWS), the Turneffe Atoll Marine Reserve (TAMR), and the South Water Caye Marine Reserve (SWCMR). These MPAs are fished by fishermen mainly from 12 coastal communities, namely: 1) Consejo Village, 2) Corozal Town, 3) Copper Bank Village, 4) Chunox Village, 5) Sarteneja Village, 6) Belize City, 7) Dangriga Town, 8) Hopkins Village, 9) Sittee River Village, 10) Riversdale Village, 11) Seine Bight Village, and 12) Placencia Village.

The Belize Marine Conservation and Climate Adaptation Project (MCCAP) has developed a programme to conduct pilot investments into repopulating reefs within replenishment zones of Turneffe Atoll Marine Reserve (TAMR) and South Water Caye Marine Reserve (SWCMR) with temperature resilient coral varieties to support climate change adaptation measures that will improve the resilience of the reef. MCCAP contracted Fragments of Hope, Ltd., to implement the reef restoration activities in TAMR and SWCMR (Sub-Component 1.2.3), and by extension to expand the reef restoration programme in Belize. With financing from the Adaptation Fund, these activities will also compliment other tasks under Component 1, such as field verification of spatial mapping activities via ground-truthing and carrying out stakeholder consultations (Sub-Component 1.2.1), and biological and water quality (temperature) monitoring of strategic and control sites (Sub-Component 1.2.2).

Additionally, Fragments of Hope will add to the project outcomes under Component 3, Raising Awareness and Building Local Capacity through Project Information Dissemination (Sub-Component 3.2.3) and Community Training Events (Sub-Component 3.2.4).

Fragments of Hope has increased live coral cover at LBCNP from just 6% to over 35% by outplanting nursery-reared acroporids from 2010-2016 in ~ one hectare of degraded reef, and is an international example of effective reef ecosystem restoration. Fragments of Hope has established replicable methodologies for mapping, genetics, outplanting and most importantly, created quantifiable success indicators for evaluating the replenishment process. This technical report addresses the site selection process and locations for coral nurseries and outplanting sites established to date in SWCMR and TAMR.

Methods:

Total Linear Extension (TLE) for *Acropora cervicornis* in nurseries

Rope culture (for *A. cervicornis* and *A. prolifera*) on table nurseries is described in detail in the Inception Report (p. 23) and also in the FoH Reef Replenishment manual housed on the FoH website[1]. Total Linear Extension (TLE, Kiel et al. 2012) is used to measure *A. cervicornis* in the nurseries, to compare growth rates between different genets (individuals) and nursery locations. Starter fragments are ~4-12 cm and measured at Day 0 when placed in the nursery. Measurements are taken ~every two months and usually do not go past six months; each new extension is measured, totaled and Day 0 is subtracted, then divided by the number of days of growth and multiplied by 30 for a monthly average growth rate. The longer the corals are in the nursery, the more extensions they have and thus monthly growth rates tend to increase over time. This method does not account for the diameter (thickness) of branches and is not as accurate as measuring buoyant weight, but is most practical for hundreds of fragments in

[1] http://fragmentsofhope.org/case-study-manuals/

multiple off shore nurseries. In general, every individual genet is set on three ropes with ~ 30 total replicates for statistics.

Hemp rope

Polypropylene ropes (1/4") have been used in FoH table nurseries since 2009. Cotton ropes were trialed but the corals snag on these ropes and they were not effective. While the acroporids have a 'pruning' vigor, meaning they grow back faster after the first harvest, experience has limited use of the polypropylene to three harvests or three years, whichever comes first. The weight of the corals and time underwater tend to make the ropes fray (the cut ends are burned upon installation, sealing them, but over time breakages can occur). Because in most cases the corals also grow over and along the ropes, FoH has usually then outplanted the ropes with corals onto the reef using cement nails (described in detail in the Inception Report pp. 25-26, and also in the FoH Reef Replenishment manual housed on the FoH website). While this method is excellent for setting a lot of corals quickly on the reef (see Figures 16a-b, e-f, 18a-b), particularly in conditions too rough to support outplanting corals with cement, concerns about the frayed rope strands causing micro-pollution continue. Hemp rope was trialed in the SWCMR nursery (March 2018) with only one replicate sourced from a manufacturer, by request, in the Netherlands.

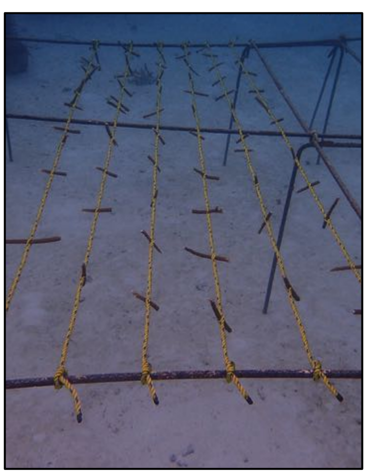


Fig. 1a-c. The standard polypropylene (1/4") rope on the left, and the hemp rope (1/2"), new, (March 2018) on the right.

Micro-fragmenting

Dr. David Vaughan formerly of MOTE Marine Laboratory[2] now head of Plant a Million Corals[3] has been involved in coral restoration since at least 2004. The focus at MOTE has always been land-based nurseries and in recent years a fortunate discovery was made: smaller fragments grow back exponentially faster than 'normal' growth rates (Vaughan *et al*. 2015)[4]. Ms. Carne visited MOTE in 2004 for attendance in a Coral Disease course, and again in 2015 for participation in a Sponge Biodiversity course. In 2015 Dr. Vaughan highlighted his results on site and gave a tour of the land-based nurseries. Nursery-grown acroporids in Belize (and elsewhere) already exhibit what is termed "pruning vigor", meaning once they are harvested/fragmented in the nurseries, they grow back even faster than the first 'generation'. However, from literature research and restoration work begun over a decade ago, the general belief was that the larger the transplanted coral, the higher its survival/success on the reef. In 2006, it was recommended that *A. palmata* transplants be at least ~30 cm, for example. Thus, since *in situ* coral nursery work began in Belize in 2009, *A. palmata* were grown in the nurseries a minimum of 12 months before outplanting.

Since Dr. Vaughan's work is land based (micro-fragments are placed in raceways with controlled conditions prior to placement on the reef), two experiments were discussed from 2015: 1) Micro-fragmenting *A. palmata* for direct placement on in –water (*in situ*) nurseries and for direct outplanting, 2) micro-fragmenting of several slower growing species (e.g. star and brain corals) that were placed in the nurseries near Placencia in 2009 and still remain in the nurseries, to compare growth rates. Dr. Vaughan was further inspired after seeing the results from work in Belize at the ICRS in HI (2016) and came to Placencia in March 2017 to share his methods with FoH (Figs. 2a-d).

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[2] https://mote.org/staff/member/david-vaughan
[3] http://plantamillioncorals.org
[4] https://peerj.com/articles/1313/?utm_source=TrendMD&utm_campaign=PeerJ_TrendMD_0&utm_medium=TrendMD

This technique has become so popular for land-based nurseries that the diamond saw is now marketed as a coral propagation tool[5], specially made in California, however (in 2017) no one had yet tried the method for direct placement in *in situ* nurseries, or direct outplanting. In March 2017 FoH and Dr. Vaughan experimented with two size classes for *A. palmata* (one-and five cm) for placement in the nurseries and directly onto the reef at the Whipray Caye nursery (near Placencia). We also tried *Orcibella annularis* (a major reef-building coral but extremely slow growing, replicates have been in nurseries since 2009) with one-and five cm size classes, in nurseries, and the five cm size class directly outplanted onto the reef. The micro-fragments were affixed to the same cement 'cookies' (discs) with superglue (Fig. 2a-d); the glue goes onto the exposed skeleton, not the live tissue. The one-cm *A. palmata* micro-fragments were placed in replicates of two to five on each cement 'cookie', to see if they would fuse and grow faster than the previous methods.

Fig. 2a-b. Dr. David Vaughan at Whipray Caye, demonstrating micro-fragmenting to FoH team members. *A. palmata* were sliced into thin strips first, then the smaller one-cm micro- fragments were made. The white is exposed skeleton, only the orange is the thin layer of live tissue.

[5] https://fragtasticreef.com/gryphon-aquasaw-diamond-band-saw/

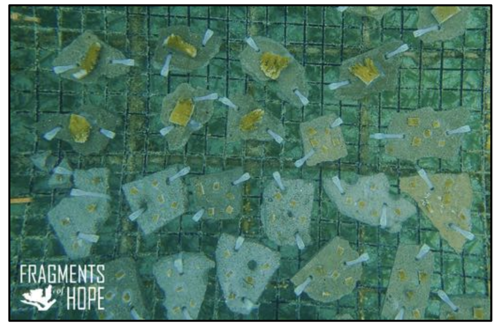


Fig. 2c-d. FoH team members placed the micro- fragments onto the cement cookies (discs) using superglue (L). Whipray caye nursery: five cm size classes (top) and one-cm size classes in replicates (bottom) (R).

Based on excellent preliminary results, 100% survival for both *A. palmata* size classes, in the nurseries and 87% survival directly outplanted after two weeks, trials were expanded to SWCMR and TAMR nurseries. The one-cm size class for *O. annularis* failed, but the 5-cm size classes had 93% survival in the nurseries and 100% survival directly outplanted (two months).

These results changed after the bleaching/disease events in 2017 (outlined in the Summary of bleaching events in Belize 2017-2019 Report) caused high mortality of the *A. palmata* micro-fragments in the nurseries, whereas the directly outplanted *A*. *palmata* micro-fragments on the shallow fore reef at SWC (plot 1, Figs. 9a-c) survived and even thrived.

As with all coral reef restoration fieldwork, modifications are continually made to improve techniques, efficiency, and results. Through a research collaboration with the University of North Carolina (UNC), FoH acquired a larger wet tile saw (Fig. 3a) which makes processing the larger fragment sizes (~5cm) much faster: the original smaller saw is more appropriate for very tiny (<5cm) micro- fragments for use in land-based nurseries. The larger saw still uses diamond-edged blades. Another modification is eliminating the cement disk substrate for the micro fragments, particularly for *A. palmata* since these are now directly outplanted in cement on the reefs. Some trials began in 2018 with *Orbicella annularis* and 2020 with *O. faveloata*, but for the pillar coral species, *Dendrogyra cylindrus*, FoH is still super gluing the exposed skeleton onto cement disks (aka "cookies") because of their extended polyps (see Figures 8a-c).

Fig. 3a. The first, recommended saw FoH trialed on the left, pictured with Dr. Vaughan, and the larger wet tile saw (still with diamond blades) FoH prefers, on the right, photo from Carrie Bow Caye in SWCMR.

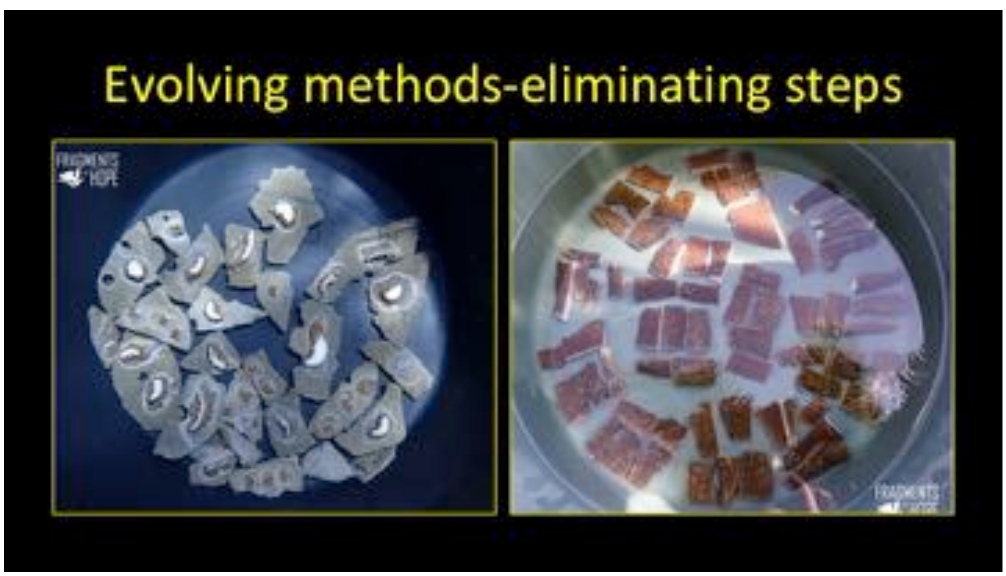


Fig. 3b. As learned from Dr. Vaughan, originally FoH super glued coral fragments (the exposed skeleton) onto cement disks for outplanting in cement (L). During transport the fragments often became dislodged from the disks, so this step was eliminated for direct outplanting of certain species into cement on the reef, pictured is *A. palmata* (L). Note also the larger fragment size.

Fig. 3c. Less than 10% of the donor/mother coral is collected for any restoration method, person in picture for size reference of pillar coral, *D. cylindrus*, L, on the shallow fore reef in SWCMR.

Results: Acropora Mapping/Donor corals

Mapping acroporids is an ongoing process, which began in 2006 and continues today. Methods are described in the Inception Report; FoH is currently exploring the use of drones to quantify the larger natural patches. The maps that follow below are data acquired through May 2020 and are separated by the three acropora taxa (*A. cervicornis*, *A. palmata* and *A. prolifera*). There are two maps for each taxa: country-wide presence including data from partners, color coded, at the University of Belize, Dr. Nicole Fogerty (UNC) and the Atlantic and Gulf Rapid Reef Assessment (AGRRA), and a second map highlighting by color the corals sourced and used in this project (some points do not show well in the larger maps, see Table I for number of different genets/individuals outplanted by taxa and MPA). These maps are evolving and expanding data sets as more acropora stands are identified by FoH, and partnerships expand within Belize (e.g. Hol Chan Marine Reserve, current students at UB) contributing additional data. See Figures 4i-ii below as example of newly found large stand of *A. cervicornis* near the Round House (RH) table site, south of Calabash Caye. Corals from RH table were added next to this stand June 2020 (different genets), in an unmeasured outplant site, the only new/ different outplant site from the Site Selection report/maps.

Figs. 4i-ii. Example of recently identified large natural stand of *A. cervicornis* in TAMR (L), and new outplant site near it, CC2 (R).

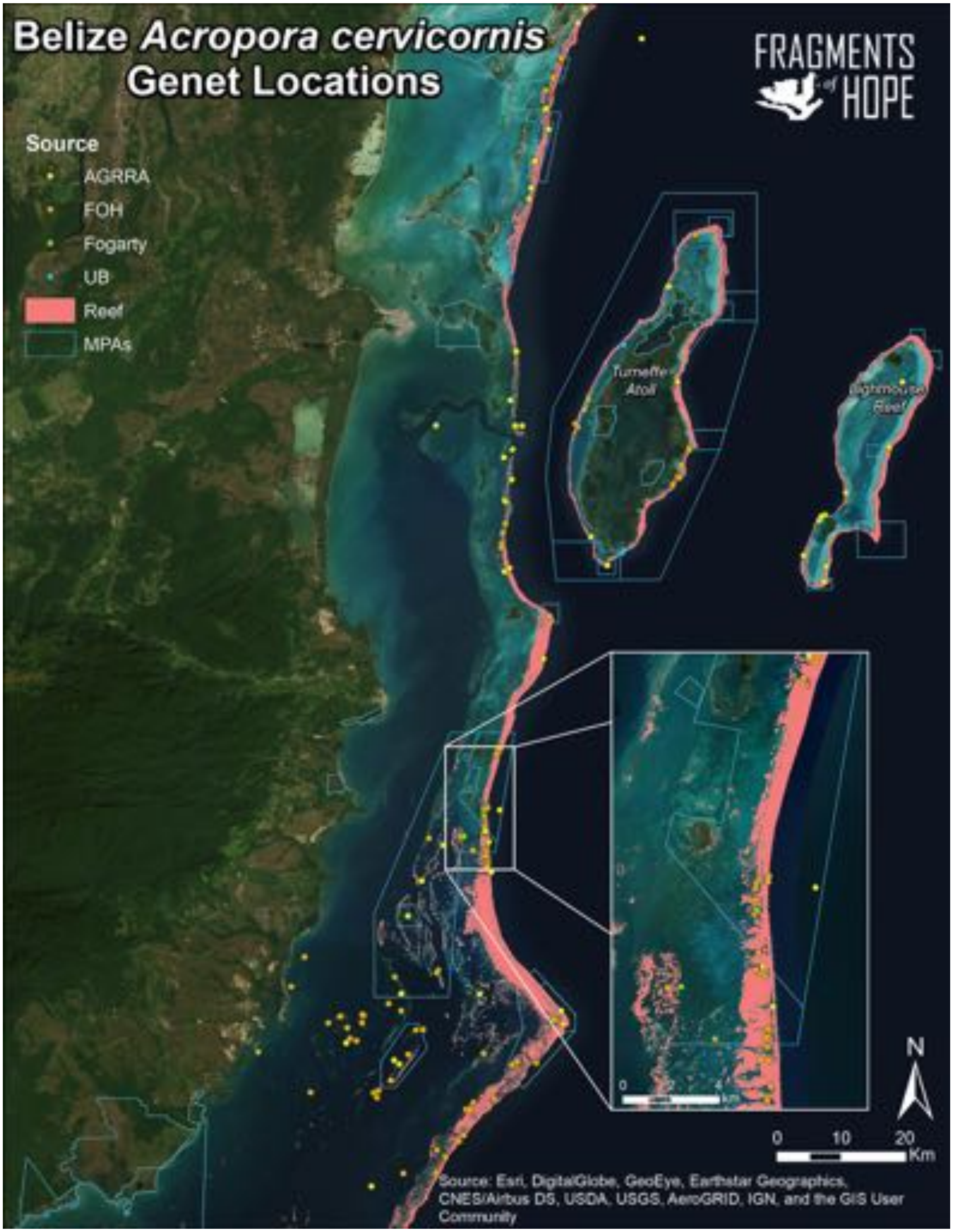


Fig. 4a. Distribution of *A. cervicornis* within Belize, color-coded by data contributing partners, the University of Belize, Dr. Nicole Fogarty (UNC) and AGRRA.

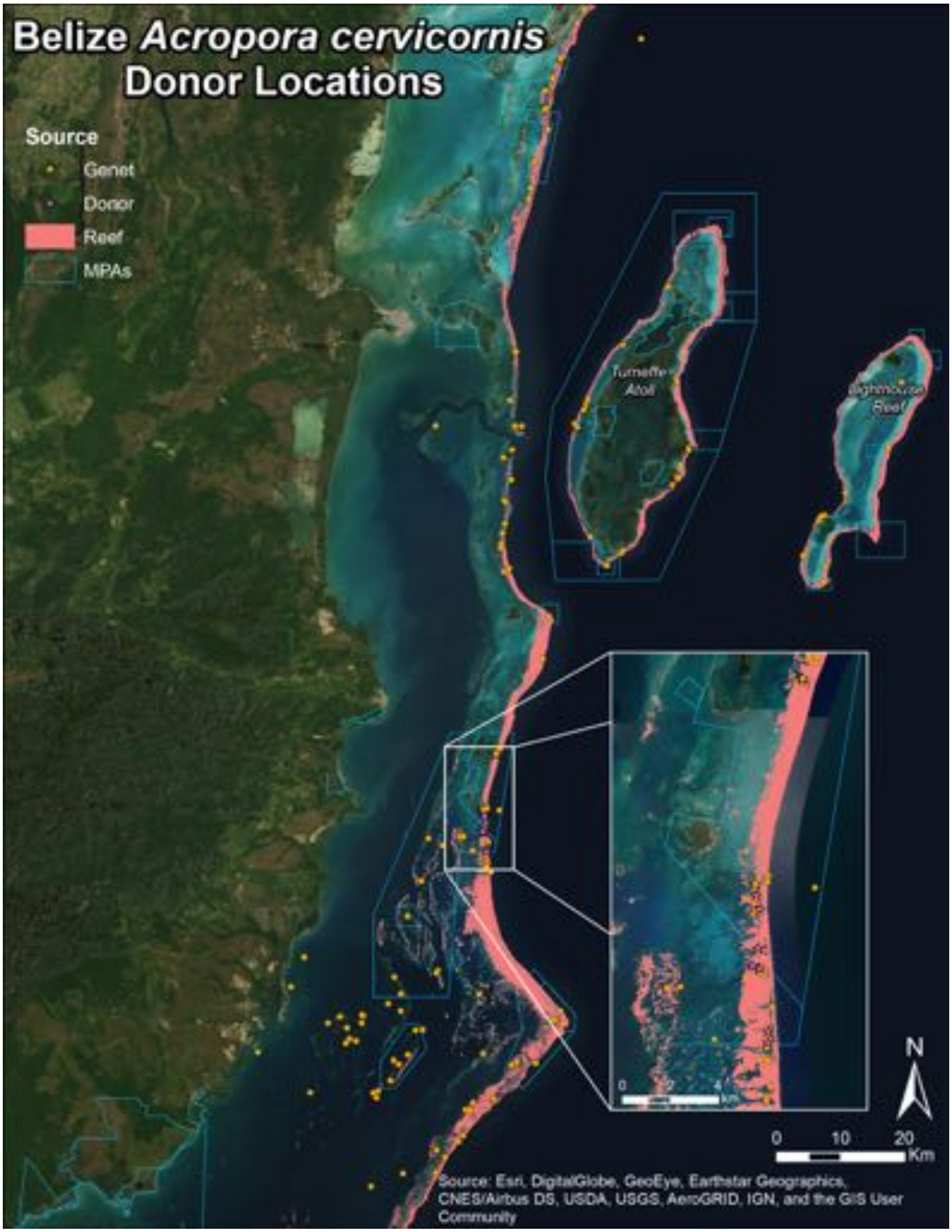


Fig. 4b. *A. cervicornis* sourced for replenishment work under MCCAP highlighted in purple stars, the points in TAMR do not show well, but 11 *A. cervicornis* genets were utilized, see Table I and Figs. 4i-ii.

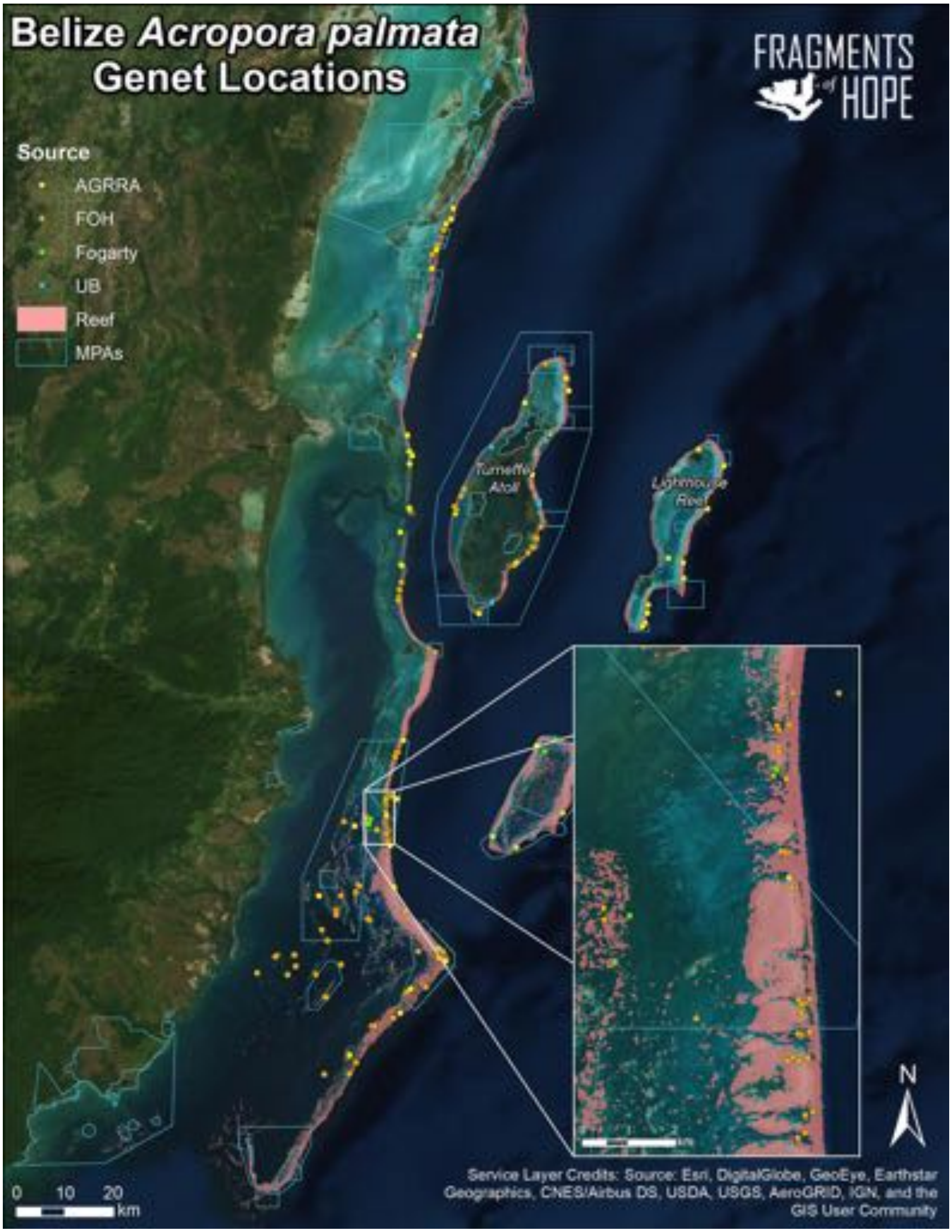


Fig. 4c. Distribution of *A. palmata* within Belize, color-coded by data contributing partners, the University of Belize, Dr. Nicole Fogarty (UNC) and AGRRA.

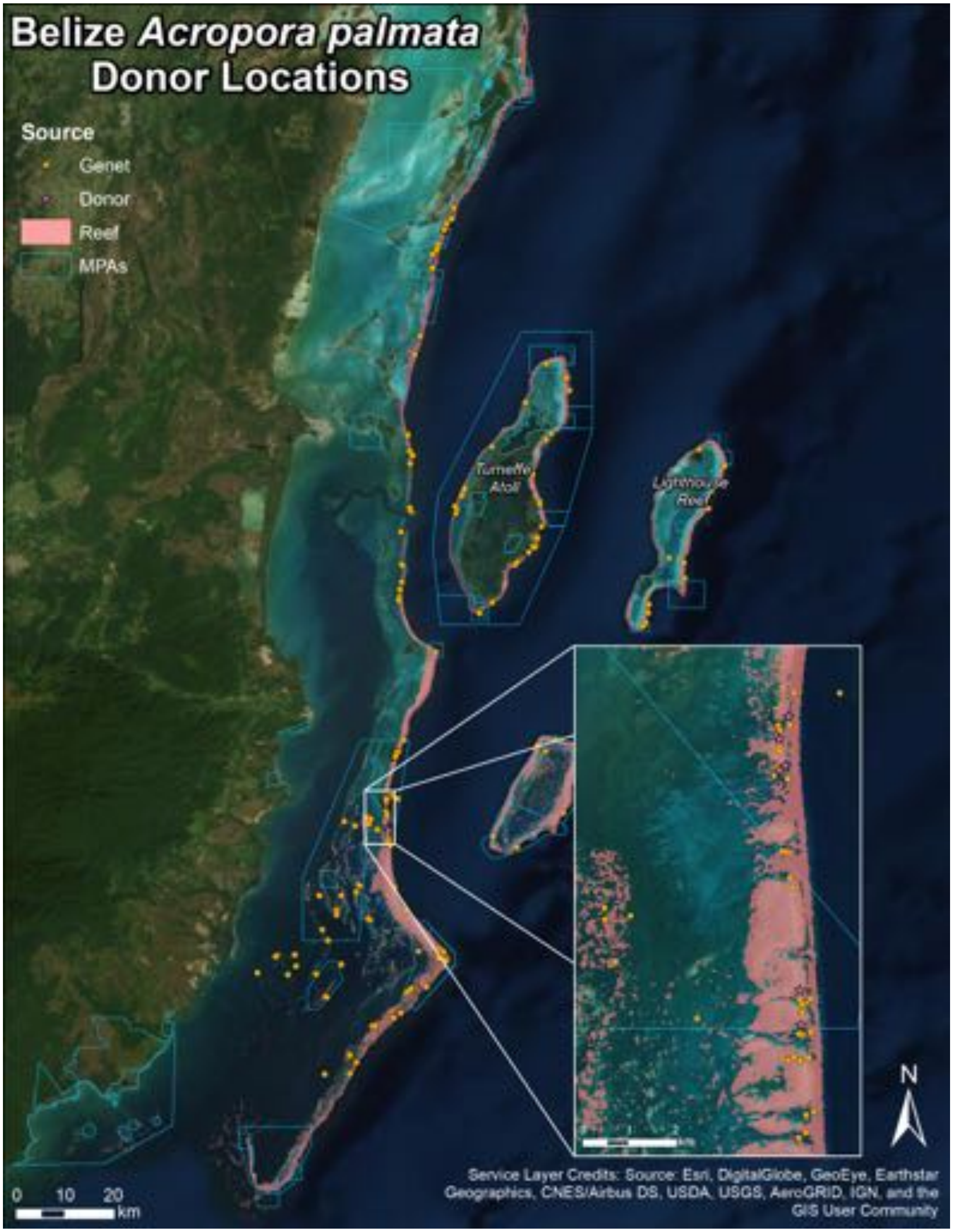


Fig. 4d. *A. palmata* sourced for replenishment work under MCCAP highlighted in purple stars, some points in TAMR do not show, but 17 *A. palmata* genets were utilized, see Table I.

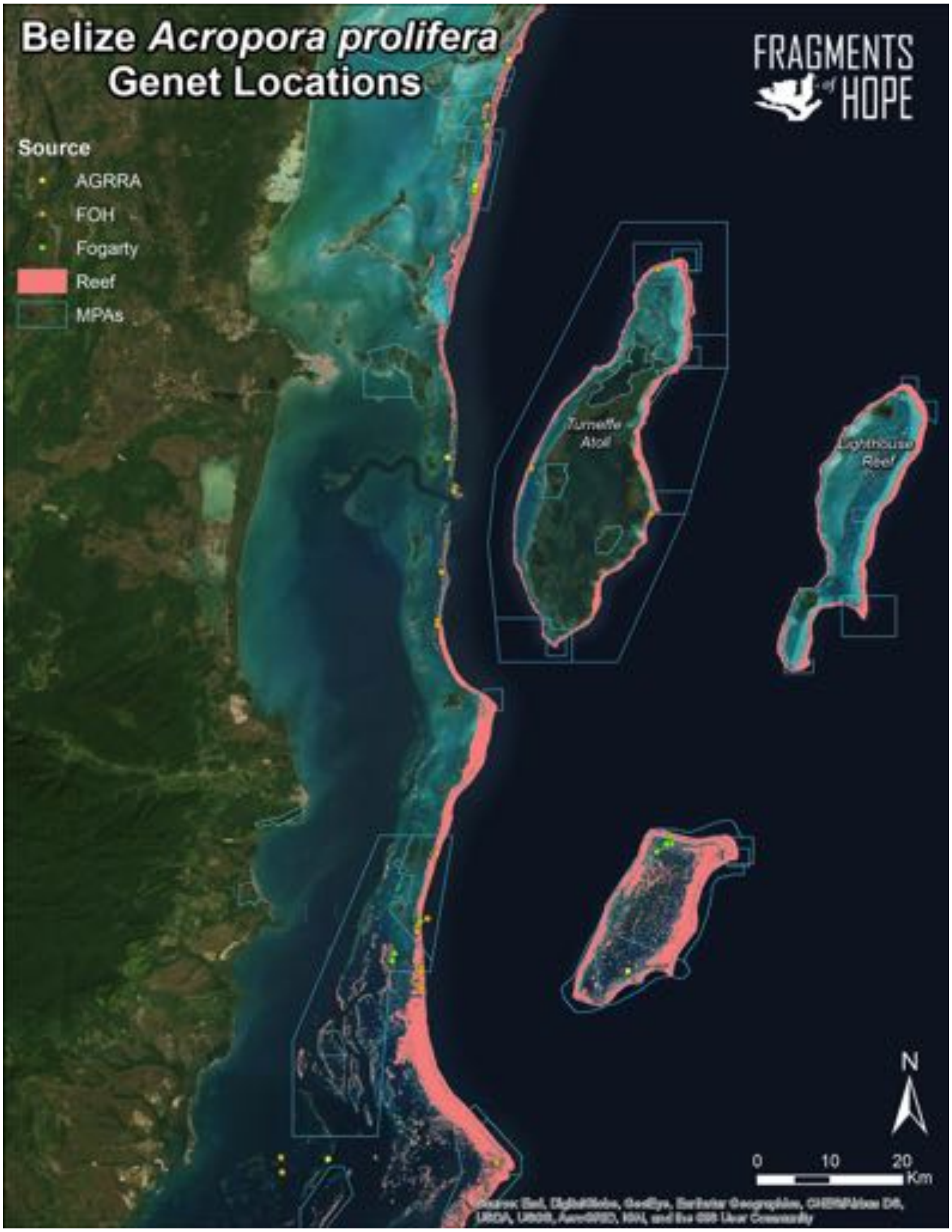


Fig. 4e. Distribution of *A. prolifera* within Belize, color-coded by data contributing partners, the University of Belize, Dr. Nicole Fogarty (UNC) and AGRRA.

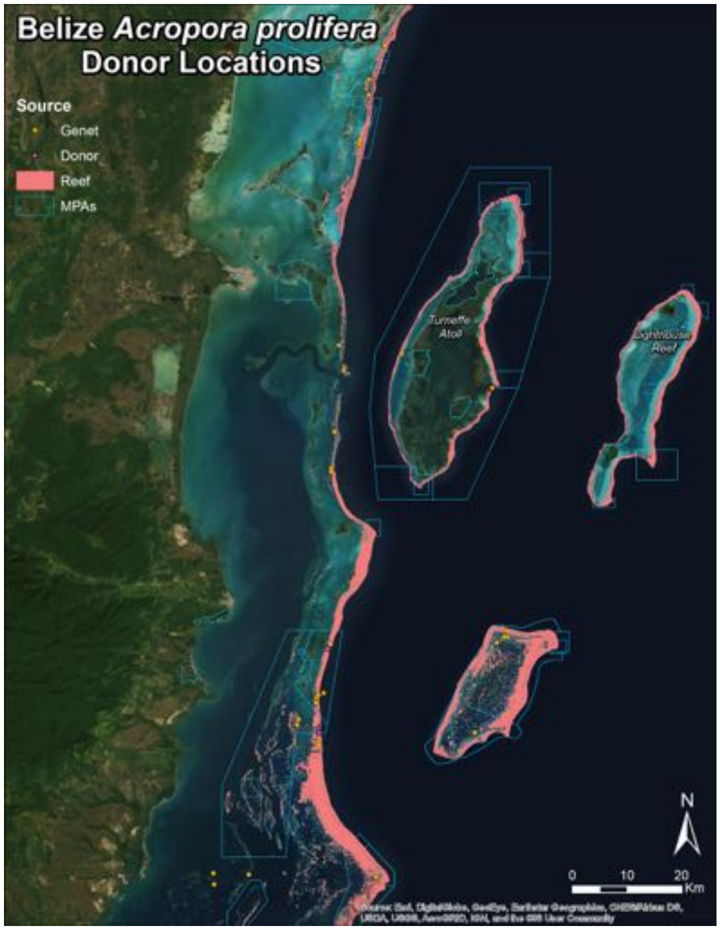


Fig. 4f. *A. prolifera* sourced for replenishment work under MCCAP highlighted in purple stars, some points in TAMR do not show, but four *A. prolifera* genets were utilized, see Table I.

Genetics:

Although 89 samples were analyzed by Dr. Baums' lab, this was primarily to ensure genetic diversity of each species/taxa was included in the nurseries/outplant sites, see Table I below (Baums et al. 2019). Since acroporids are hermaphrodites but cannot self-fertilize, genetic diversity is crucial to support successful cross-fertilization when spawning of outplants occurs (Carne and Baums 2016). However, the real desire to link genetic and phenotypic traits, in order to select corals that may be most resilient to bleaching and/or disease events, and/or grow very fast, as described in the Inception Report (2016), was not readily accessible as yet- "interest outpaced primary research" (Parkinson et al. 2020). Since 2018, a new technology was developed, "SNPchip" (SNP=Single nucleotide polymorphism) and this 'microarray' uses > 30,000 SNPs for the acroporid family[6]. FoH has extended the agreement with Baums' lab and ~128 samples are prepped and ready for this new analysis, but results will be two-three months. The full technique is described in Kitchen et al. (2020).

Table I. Tally of number of different genets outplanted for each species/taxa in each MPA.

MPA	Species	Number genets outplanted	MPA	Species	Number genets outplanted
TAMR	*A. palmata*	17	SWCMR	*A. palmata*	15
TAMR	*A. cervicornis*	11	SWCMR	*A. cervicornis*	9
TAMR	*A. prolifera*	4	SWCMR	*A. prolifera*	2
			SWCMR	*D. cylindrus*	2

Results: *A. cervicornis* growth rates (TLE), survivorship and hemp rope

Staghorn: Survival rates for *A. cervicornis* in both SWCMR and TAMR nurseries were ~80-100% for over 200 days (Fig. 5d). The TLE growth rates tell a more detailed story (Figs. 5a-c). Figure 5a shows the nursery sites color-coded in the legend and graph, with the genet source on the X-axis, with south to north illustrated left to right. The slowest growth rates are at Calabash Caye table 2 (CC2, bright orange in the legend) and Black Bird Caye table 2 (BBC2,

[6] https://news.psu.edu/story/629300/2020/08/24/research/new-tool-identifying-endangered-corals-could-aid-conservation

pink in the legend). The highest growth rates are found on the shallow Tobacco table 1 (dark green) and South Water Caye table 3 (light green). Two obvious conclusions can be made from this data, the first being that *the TAMR corals grew markedly slower* than the SWCMR corals, and when compared to existing data sets from southern Belize (Fig. 5b) the trend is even more obvious: the further south and (to some extent) west the corals are sourced and placed, the faster they grow. This meant that SWCMR *A. cervicornis* were ready to outplant before the TAMR *A. cervicornis*, with Calabash Caye table2 being the most extreme example (two years in the nursery before harvesting).

Figure 5b shows the source of the many genets from the three areas in Belize, arranged from south to north (left to right) on the x-axis with the TLE averages (cm/month) for southern Belize (12.5cm/month), SWCMR (6.1 cm/month) and TAMR (2.6 cm/month) in black bars after their sourced genets. Figure 5c illustrates this trend on a map. There was no correlation with temperatures, but one hypothesis suggests near shore corals may benefit from more nutrients. This warrants further study. It should be noted that there is nothing "wrong" with the slower growing corals in TAMR, it simply means readjusting the timelines and expectations for reef replenishment results in TAMR.

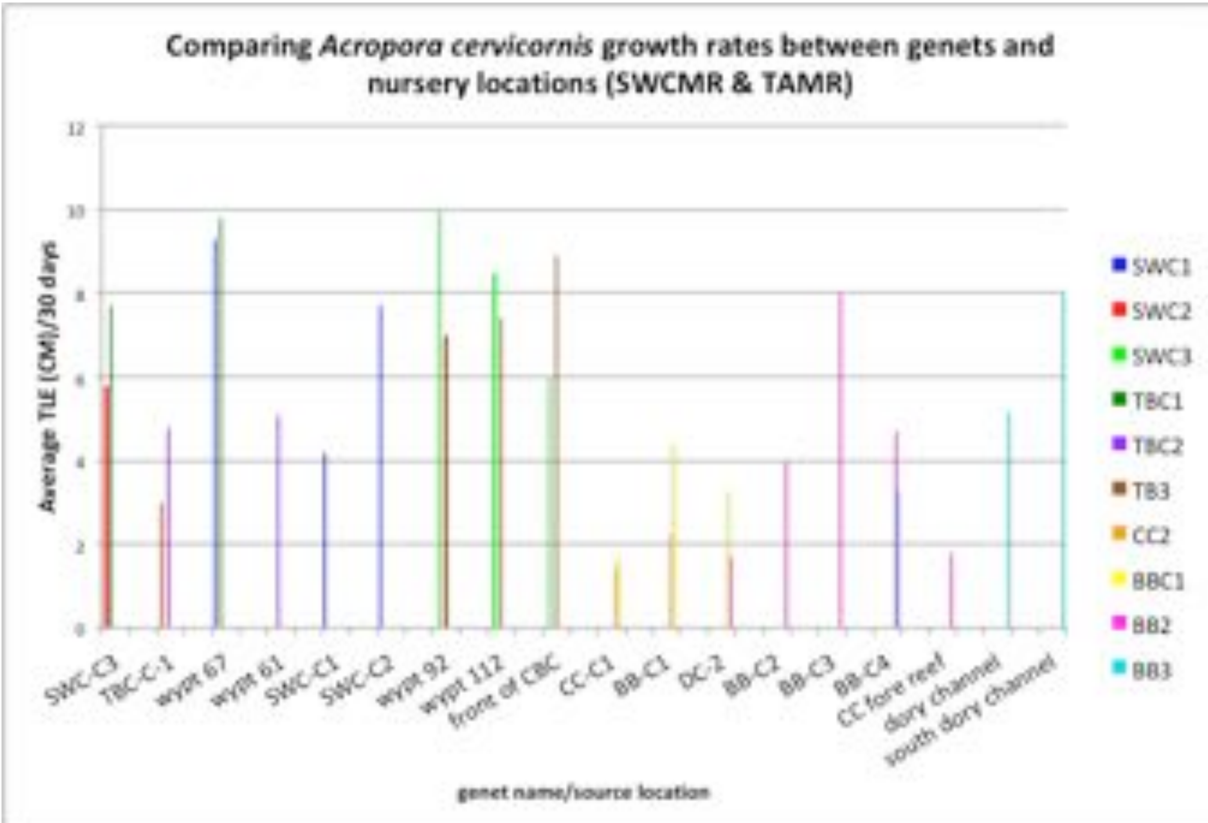


Fig. 5a. Comparing *A. cervicornis* growth rates (TLE cm/month) across genets (x-axis) and nursery locations (legend), in both MPAs. Replicates are ~ 30 for each genet.

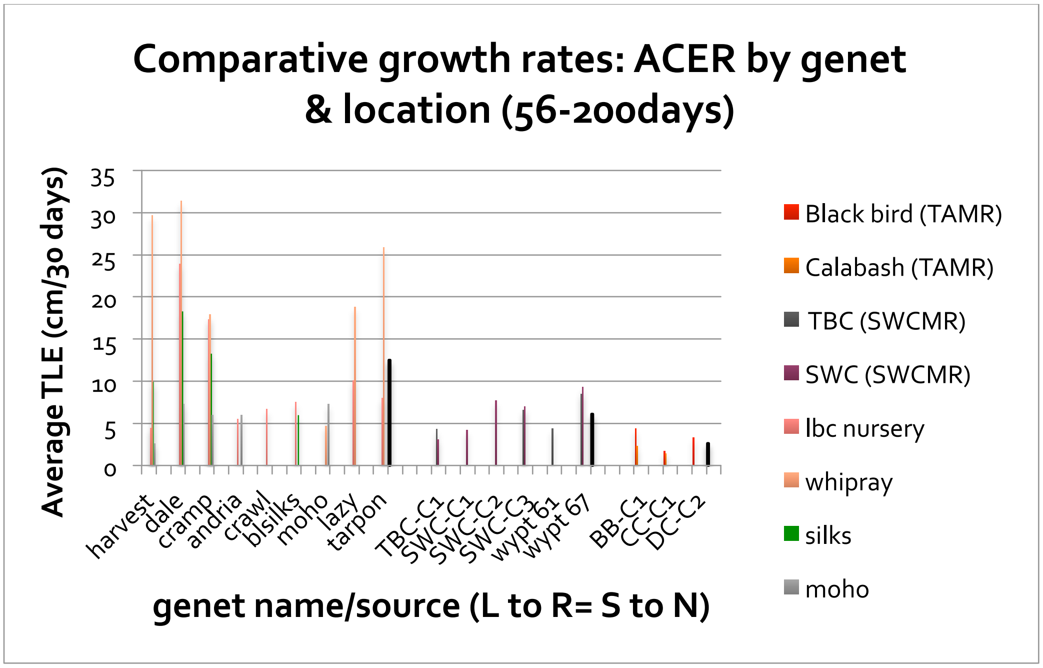


Fig. 5b. Comparing *A. cervicornis* growth rates from south to north (left to right on x-axis). The three regions (southern, central and northern) averaged TLE (cm/month) are shown in black bars.

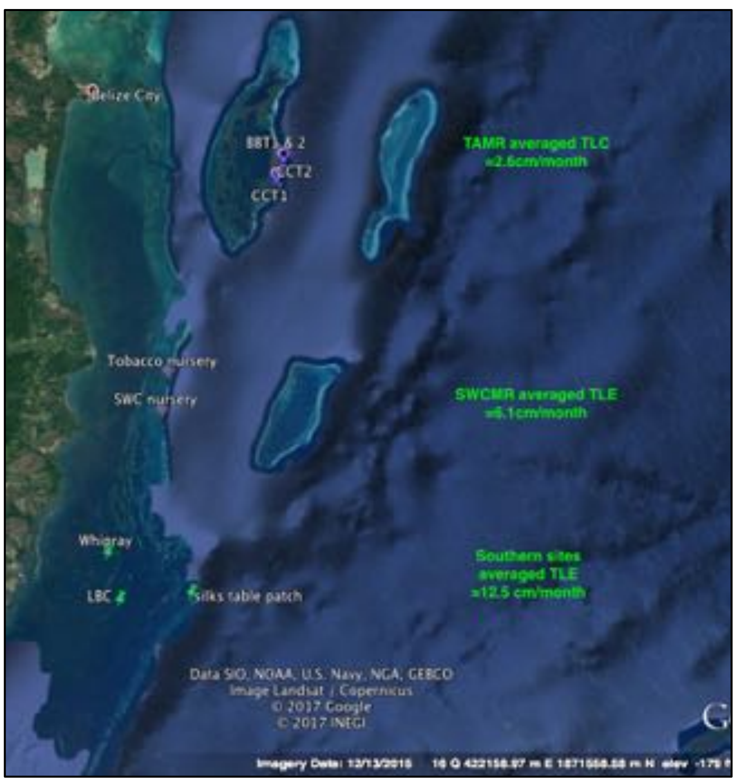


Fig. 5c. Map with the averaged TLE (cm/month) for *A. cervicornis* in nurseries from north (2.6cm/month) to central (6.1cm/month) to south (12.5cm/month).

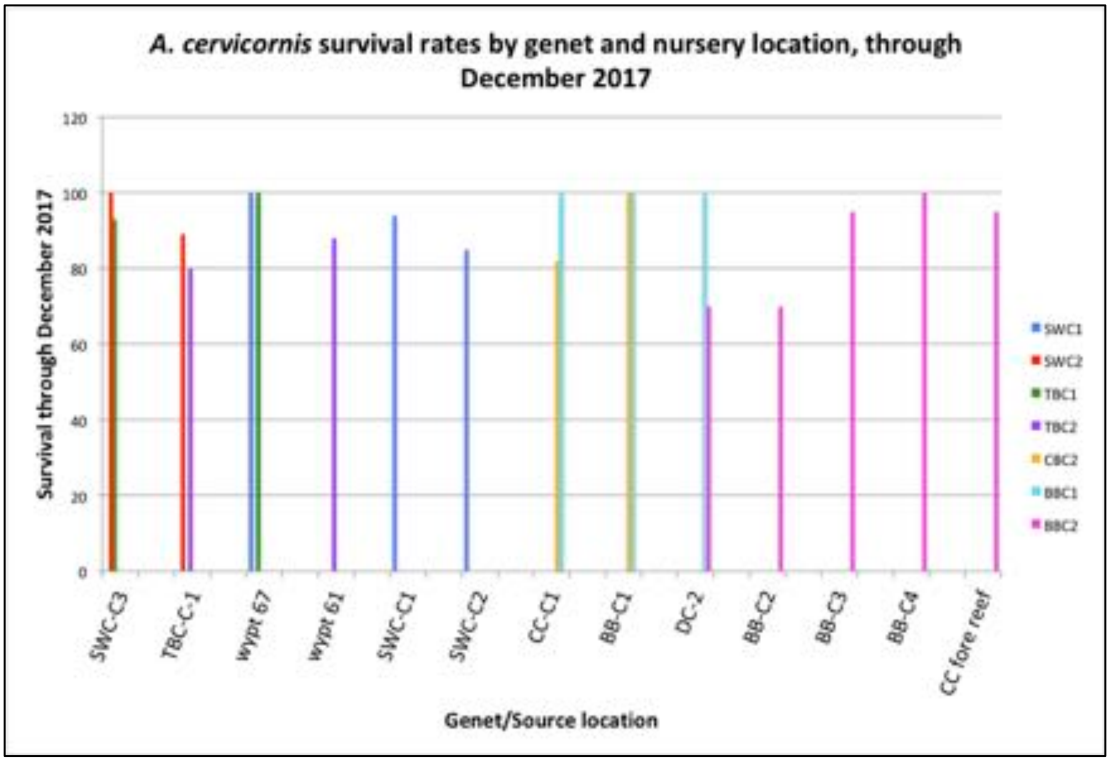


Fig. 5d. Comparing *A. cervicornis* survivorship across genets (x-axis) and nursery locations (color coded, legend), in both MPAs. Replicates are ~ 30 for each genet and percent survivorship is through December 2017.

Elkhorn: There was variability in survivorship by nursery location, genet (Figs. 6a-b), and to some degree, fragment size (Table II). Corals sourced from and placed in Black Bird Caye sites had higher survival and growth rates than the corals sourced from and placed at Calabash Caye in TAMR: thus, the last nursery was placed in a new location, "Round House reef" south of Calabash Caye (Fig. 4ii). While historically the *A. palmata* had much higher survival rates in the southern sites than those listed in Table II, in previous years, those corals had not experienced the severe temperatures of 2017 (or 2019). Because the trial SWC *A. palmata* micro-fragments placed directly on the shallow fore reef (Figs. 9a-c) had better survival rates than those in the nurseries, placement of *A. palmata* in nurseries was discontinued, with a focus instead of directly outplanting micro fragments onto the reef 2018-2020.

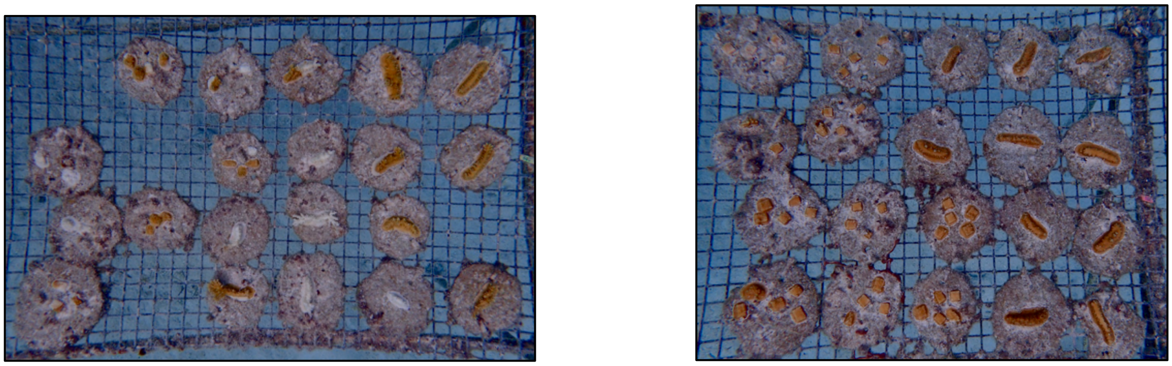


Fig. 6a-b. Most of the *A. palmata* micro fragments in the nurseries had high mortality in the 2017 bleaching event although random mortality pattern on the same genet (L) suggests rapid disease, perhaps transferred by a fish (vector). Note definitive line (s) on some corals. Some genets survived (R) from BBT2, December 2017.

Table II: Elkhorn survivorship in the nurseries after 2017 bleaching/disease event:

Nursery	method	Total number	% surviorship
TBC Table1	cookies	22	32
TBC Table 2	cookies	25	8
TBC Table 2	Micro-frags	83	16
SWC Table 1	cookies	36	14
SWC Table 2	cookies	15	0
SWC Table 2	Micro-frags	88 and 90	1 and 58
CC table 1	cookies	29	51
CC table 1	Micro-frags	188	27
BB table 1	cookies	20	55
BB table 2	Micro-frags	184	66

Hemp Rope: The single trial hemp rope degraded after less than eight months underwater (Fig. 7a), and even more disappointing was the fact that the corals never could grow over the rope (Figs. 7b-c), as they do with the polypropylene ropes and in some cases seemed to kill the coral. Practitioners noted it was extremely difficult to clean the fouling algae on the hemp ropes, and there was more of it, then on the polypropylene ropes. FoH will research other biodegradable options as since reporting these results to the wider restoration community, it was learned there are several grades and types of hemp rope that could be of superior quality, which warrants further exploration.

Figs. 7a-c. The hemp rope broke after ~eight months (L) and the corals could not grow over the hemp rope (center and right), as they usually do on the polypropylene ropes.

Results: Replenished Sites

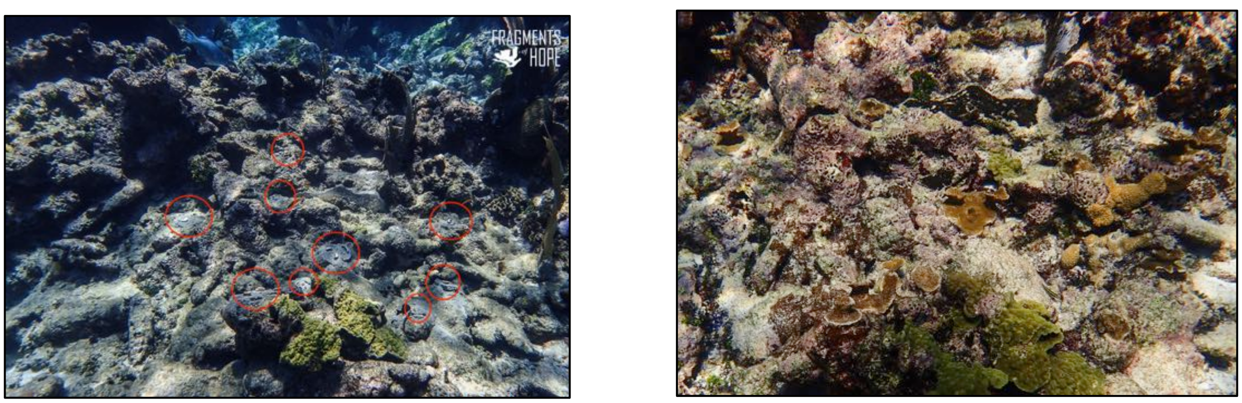
The first outplanting occurred in 2017: directly outplanted *A. palmata* micro fragments onto the shallow fore reef at SWC plot1 (Figs. 9a-c) have the longest time on the reef, under this program, over three years. One *A. cervicornis* rope from a previously installed table near Calabash Caye was also outplanted in 2017 (November, Figs. 18a-b) using no nails, just set on the shallow back reef. Figures 16c-d illustrate that when corals are wedged properly into dead reef, no epoxy, cement, or cable ties or nails are needed. This applies to ropes that are heavy enough (large enough corals, strong genets and not brittle) that may be simply set in high relief areas. More commonly, cement nails are used to fix the rope tightly to the dead substrate, to allow the corals to grow into place. The last outplanting occurred June 2020 at a new site in TAMR, near Round House Reef (Figs. 4i-ii) and large amounts of natural *A. cervicornis*.

A total of 28,927 corals fragments were outplanted across the two MPAs, the details are broken down by date, species, and areas in Table III. Most of these were acroporids with two exceptions: pillar corals (*Dendrogyra cylindrus*) were micro fragged (N=37, two different genets) in 2018 and placed in the SWC nurseries for one year with 100% survival. These were outplanted in 2019 to a shallow channel in front of the SWC tables and as of July 2020 had 91% survival, 16 months (Figs 8a-c).

Micro fragmenting and direct outplanting of *Orbicella annularis* was trial in TAMR near the Black Bird Caye tables in 2018 (N=55) and they had good survival up until the 2019 bleaching event, zero survived.

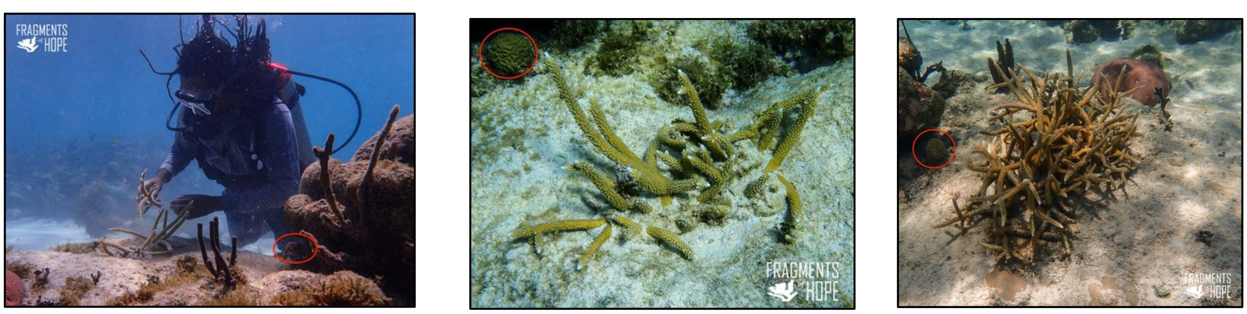
There are four measured plots in each MPA (Table IV) totaling almost 1400m^2. Once those plots are outplanted, no further corals are added, as each of these plots were photo-mosaiked prior to outplanting, and once a year after outplanting to track natural changes in replenished coral cover. None of these mosaics have yet been processed or annotated, so in the absence of that data time series photographs are shared below illustrating each outplant method (micro fragmenting, planting with cement, outplanting ropes, wedging corals) with examples from each MPA and shallow reef habitat (fore reef, back reef, reef crest). In SWCMR, 3,861 coral fragments were outplanted inside the measured plots (~649m2), meaning the majority of outplants (14,377) are outside of these measured plots. In TAMR, of the 10, 690 corals outplanted there, 3, 195 are inside the measured plots (~733m2), meaning 7,494 corals are outplanted outside of the plots.

Fig. 8a-c. Example of the pillar corals *(D. cylindrus*) that were micro-fragged (n=37) in 2018, left in the SWC nursery one year (100% survival), and outplanted in March 2019 (L). Center photo from July 2020: 16 months on the reef (shallow channel in SWC, ~5m), with 90% survival. Corals were placed near natural pillar corals stands, different from where they were sourced (R).

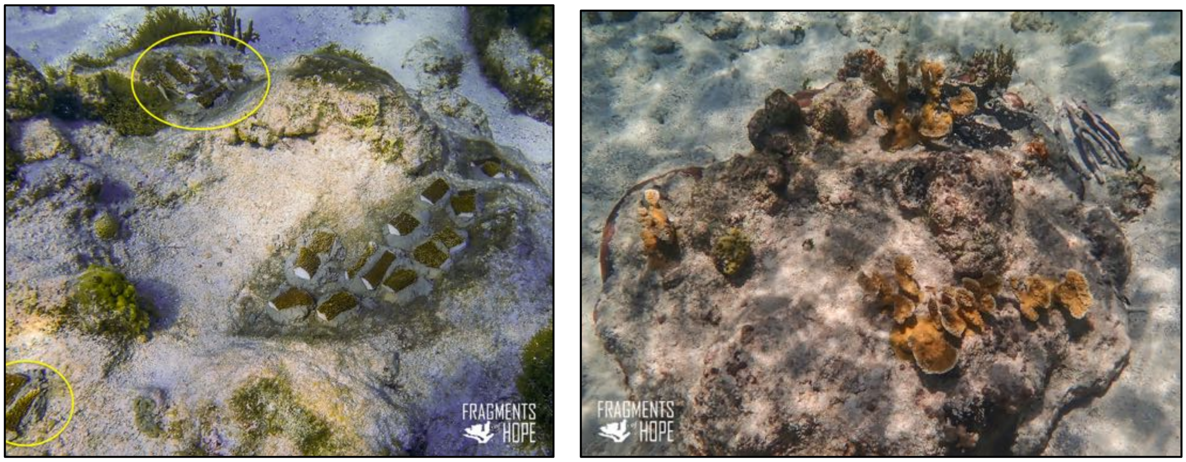


Figs. 9a-b. Examples of the first *A. palmata* micro-fragments directly outplanted to shallow fore reef at SWC in April 2017. Day 0 (L) and Day 585 (R).

Figs. 9c. Examples of the first *A. palmata* micro-fragments directly outplanted to shallow fore reef at SWC, photo from July 2020 (Day 1193 or three years and three months on the reef). Meter bar shown is in 10cm increments (black and white).



Figs. 10a-c. Nursery-grown *A. cervicornis* outplanted in cement by Jamaican exchange restoration practitioner, Inilik Wilmot, SWC back reef (outside of SWC plot 3) day 0 in 2018 (L), at three months (center) and 27 months (small brain coral circled for reference point.



Figs. 11a-b. *A. palmata* micro fragments outplanted directly in SWCplot3, shallow back reef, Day 0 in 2018 (L) and ~29 months later (R).

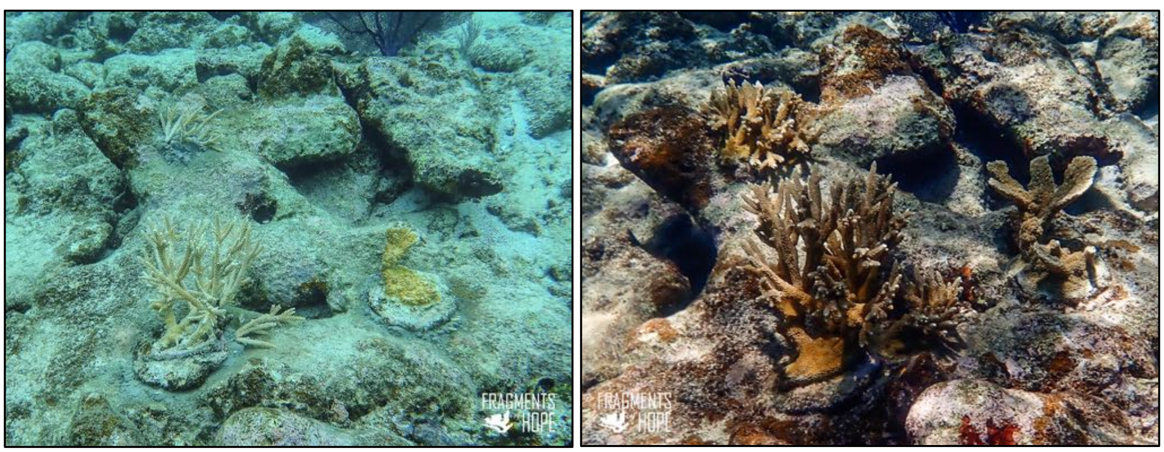
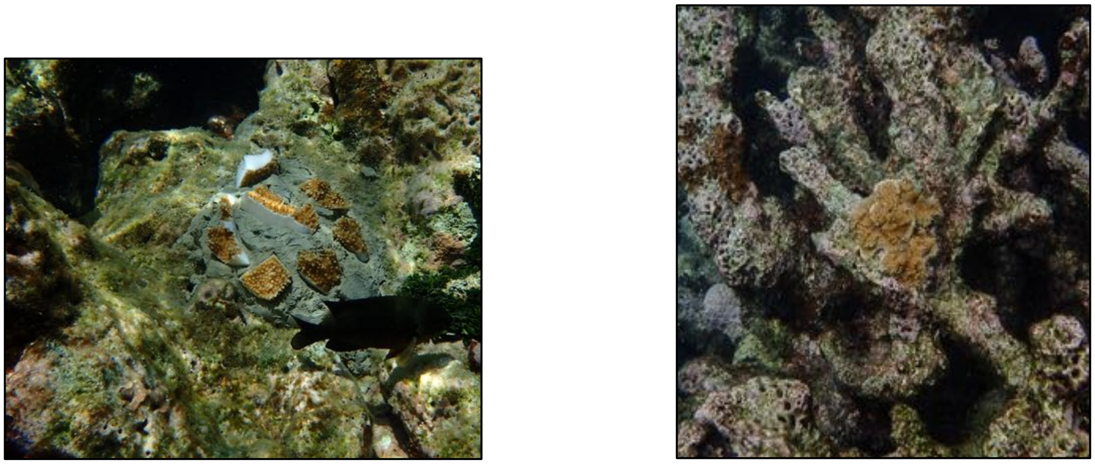


Fig. 12a-b. *A. prolifera* and *A. palmata* nursery grown corals outplanted onto the shallow fore reef at Tobacco Caye (TBC3, unmeasured) , April 2019 (L) and in July 2020 (R). Outplant site TBC3 (not a measured plot)

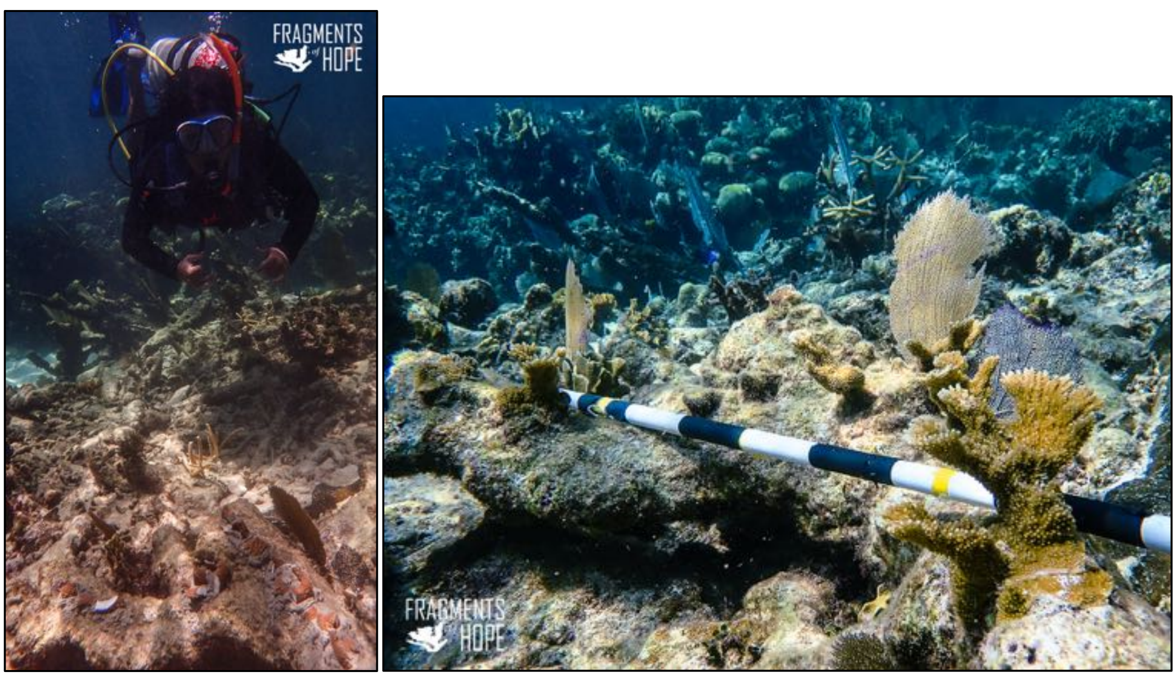
➔16 months

Figs. 12 c-d. Example of how micro fragments from the same genet quickly fuse and from a colony Day 0 (L) and just 16 months later in July 2020 (R).

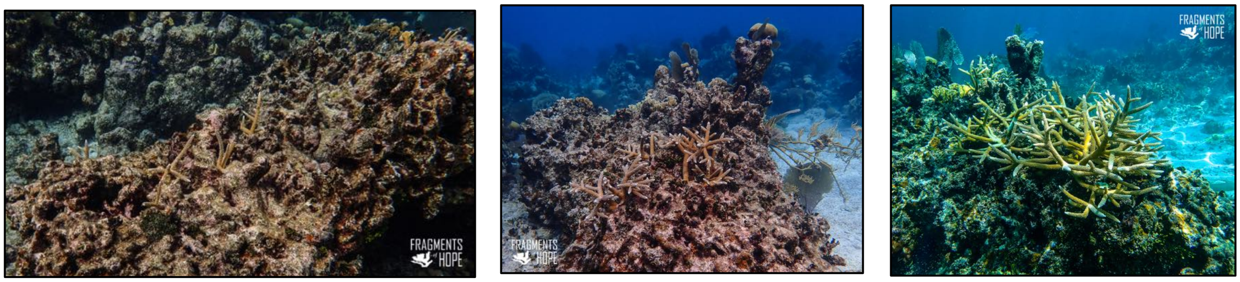
Figs. 13. a-b. Nursery grown *A. prolifera* (circled) outplanted with cement one month, and *A. cervicornis* Day 0 in 2018 at Tobacco Caye plot1 (L) and less than 2.5 years later, in July 2020 (R).



Figs. 14a-b. Example of fused *A. palmata* micro-fragments directly outplanted at BBCplot 2, a reef crest in TAMR, May (L) to November 2018 (R).



Figs. 14c-d. Day 0 outplanting *A. palmata* micro fragments directly (May 2018, L) and in July 2020 (R) in Black Bird Caye plot 1, TAMR.



Figs 15a-c. Example of 'wedging' *A. cervicornis*, Day 0 in 2018 (L) one year later (center) and two years later (R), Black Bird Caye plot 3, shallow fore reef.

Figs. 16a-b. Entire rope outplanted with cement nails May 2019 (L) and in July 2020 (R) shallow back reef at Black Bird Caye, TAMR –outside of any measured plot.

Figs. 16c-d. Close up of how secure 'wedging' and proper placement of ropes allows *A. cervicornis* to grow onto the substrate. At Black Bird Caye shallow fore reef (L) and back reef (R), pictures from July 2020.

Figs. 16e-f. This genet was sourced from northern TAMR: entire rope outplanted with cement nails May 2019 (L) and July 2020 (R) shallow back reef at Black Bird Caye, TAMR –outside of any measured plot.

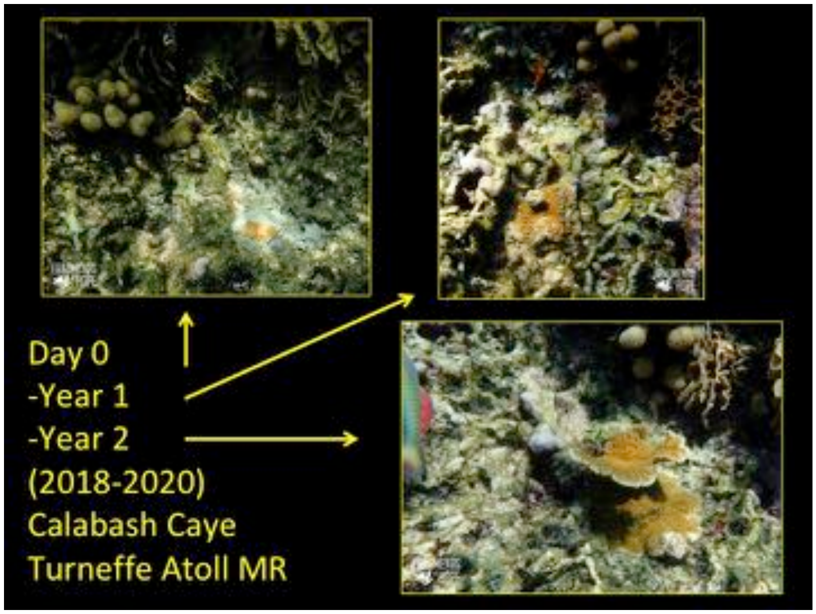


Fig. 17a. Time series of a single *A. palmata* micro fragment directly outplanted on the shallow fore reef at Calabash Caye, TAMR (CCplot 1) in May 2018, one year later (2019) and two years later (2020).

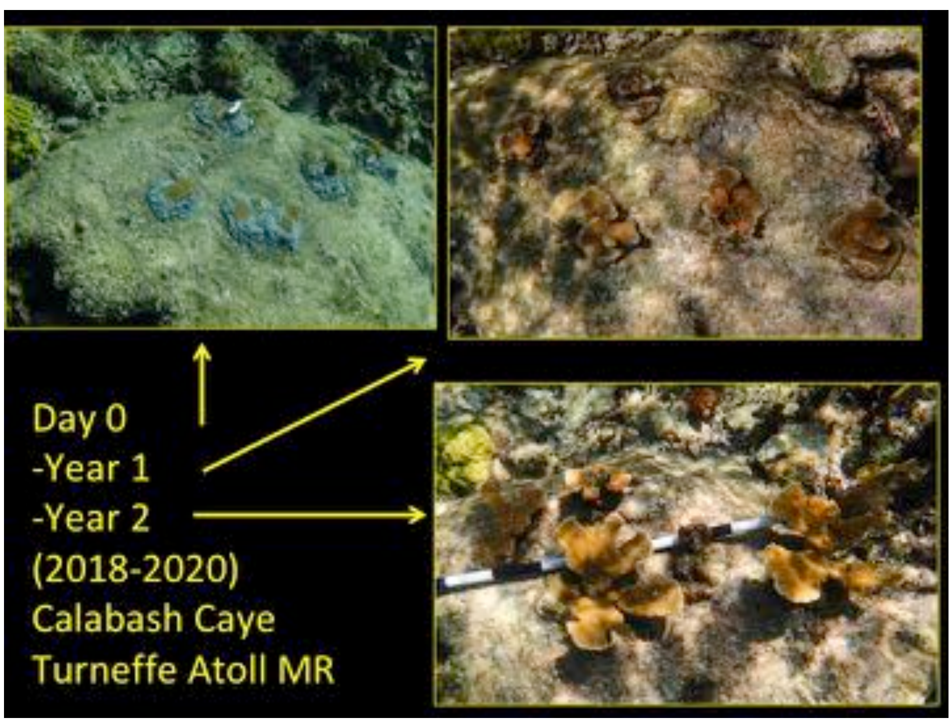


Fig. 17b. Time series of a single and multiple *A. palmata* micro fragments directly outplanted on the shallow fore reef at Calabash Caye, TAMR (CCplot 1) in May 2018, one year later (2019) and two years later (2020).

Fig. 18a-b. Example of entire rope outplanted (no nails, just set down) in back reef of Calabash Caye, set in November 2017, pictures are from May 2018 (L) and February 2020 (R).

Table III. Details of the number of outplanted corals by year, MPA, site within the MPA and species or taxa. Note: "outside of plot" is a total count of corals that may be in many smaller sub-sites in each MPA.

YEAR	SITE	SPECIES					TOTAL
		ACER	APAL	APRO	OANN	DCYL	
2017	SWC Plot 1		61				61
2018	SWC Plot 1	787	167	0			954
	SWC plot 2	544		35			579
	SWC plot 3	243	100				343
2018	SWC outside of plots	1,199		23			1,222
2019	SWC outside of plots	2,347	60	215		37	2,659
2020	SWC outside plot 1	921					921
	Sub-total SWC	6,041	327	273			**6,641**
2018	Tobacco plot 1	1,332	0	592			1,924
2018	Tobacco outside of plots	2,661		104			2,765
2019	Tobacco outside of plots	1,747	342	1,086			3,175
2020	Tobacco outside of plots (fore reef)	1,583		2,150			
	Sub-total Tobacco	7,323	342	3,932			**11,597**
	Sub-total SWCMR	13,364	669	4,205			**18,238**
2018	BLACK BIRD plot 1	1,369	378	200			1,947
	BLACK BIRD plot 2		412				412
	BLACK BIRD plot 3	749					749
2018	Black Bird outside of plots				55		55
2019	Black Bird outside of plots	2,238		1,083			3,321
	Sub-total Black Bird Caye	4,356	790	1,283			**6,429**
2018	Calabash plot 1	198	159				357
2018	Calabash outside of plot	241					241
2019	Calabash outside of plot	1,120	9	561			1,690
2020	Calabash outside of plot	898					
	Sub-total Calabash Caye	2,457	168	561			**3,186**
2020	new sites south of Calabash	1,074					
	sub-total TAMR	7,887	958	1,844			**10,689**
TOTAL SWCMR + TAMR 2017-May 2020							**28,927**

Table IV. List of the permanent, measured plots in each MPA with total area (m^2), number of each species outplanted and date outplanted.

MPA	plot name	plot area (m2)	reef type	# Acer	#Apal	#Apro	Month/Year planted
SWCMR	SWC 1	143.42	fore reef	787	228		April 2017 & March 2018
SWCMR	SWC 2	195.21	back reef	544		35	Jan-18
SWCMR	SWC 3	120.42	back reef	243	100		Mar-18
SWCMR	TBC 1	189.66	channel	1332		592	Feb-Mar 2018
TAMR	BBC 1	295.73	fore reef	1,369	378	200	May-18
TAMR	BBC 2	187.09	reef crest		412		May-18
TAMR	BBC 3	75	fore reef	749			May-18
TAMR	CC 1	175.11	fore reef	198	159		May-18
	Total	**1381.64**					

Table IV. GPS coordinates (Datum=WGS84,) for coral outplant sites established.

MPA	Plot number	Depth	Reef type	Latitude	Longitude
SWCMR	SWC 1	2-5m	Fore reef	16.48698	-88.04790
SWCMR	SWC2-3	2-4m	Back reef	16.88923	-88.06466
SWCMR	SWC4	4m	Channel	16.8110	-88.08126
SWCMR	TBC1	3m	Channel	16.89588	-88.06238
SWCMR	TBC2	3m	Channel	16.89033	-88.06462
SWCMR	TBC3	2-3m	Fore reef	16.89673	-88.05973
TAMR	BBC 1	2-5m	Reef crest	17.31781	-87.79266
TAMR	BBC2	1m	Reef crest	17.31791	-87.79295
TAMR	BBC3	4-5m	Fore reef	17.31803	-87.79236
TAMR	BBC4	2m	Back reef	17.31799	-87.79340
TAMR	BBC5	2m	Back Reef	17.31925	-87.79328
TAMR	CC1	3-4m	Fore Reef	17.27860	-87.80641

Conclusions & Recommendations

Micro fragments: In addition to the modifications described under methods, outplanting with more than one genet (together) was trialed, based on natural *A. palmata* genetic diversity within stands. While this revealed differences in bleaching, and even predator preferences (snails eating one genet but not the other), it is recommended clusters be kept separate by genets. Although single micro fragments outplanted often did well, a small cluster is best in case a few perish. This method has proved so successful, north to south, and in each shallow reef habitat, this is the recommended practice for rapid shallow reef replenishment for *A. palmata*. Current (and future) mapping reveal enough natural stands in both MPAs that choosing multiple donor corals for each target site will eliminate any nursery time and ensure genetic diversity (aiming to add at least four-six new genets to target sites). Small trials with pillar coral (*D. cylindrus*) are promising, and since this is one of the most susceptible species to Stony Coral Tissue Loss Disease (SCTLD), and one of the more-rare coral species, mapping of this species and continued trials with micro fragmenting and outplanting different genets near natural colonies should be continued. Trials with additional species should continue.

Rope-culture: This method still works well for rapidly increasing volume of *A. cervicornis* and *A. prolifera*. Hemp rope options should be explored, since outplanting corals on ropes is a rapid and efficient way to replenish large amounts of corals. Three tables were left in TAMR at the request of Boston University partners, two at Calabash and one at Black Bird Caye. In SWCMR, two tables remain near SWC and one at Tobacco Caye. All of the remaining tables can also be used for experiments with micro-fragmenting additional species.

Quantifying coral cover: Because external partners at the University of Miami have been processing the photo-mosaics, for southern Belize as well (2014-2018), there has been a time lag for results. A solution is sourcing the hardware, software for use in country, and building capacity locally for processing and annotating the diver-based photo-mosaics. The use of drones has been explored successfully in southern Belize for quantifying the amount of replenished acroporids in the shallow fringing reef at Laughing Bird Caye National Park (LBCNP); this also requires processing software and annotation, and recommendations are the same for diver-based mosaics: in country hardware, software and training.

Surviorship: Predation by snails and fire worms (for the acroporids) remains a high mortality issue, more so than bleaching and disease. Since the Replenishment (No Take) Zones in both SWCMR and TAMR are relatively new, relative to LBCNP for example, planting more corals and continued enforcement to improve the ecological balance (food chain) is important, since it is believed lobsters and certain fish are the natural predators of the snails and fire worms.

While this program was intended to be five years, in reality it was just over three years; ideally reef replenishment programs should be at least five years; for example, the expectation of the oldest outplants (in SWC) to spawn may not be until 2021 or 2022. What this program successfully established is that ***the methods for reef replenishment in use in southern Belize for over a decade (Carne et al. 2016) are replicable throughout Belize.*** Much of the fieldwork accomplished in this program was with four-eight team members, who also maintained and outplanted multiple other sites outside of SWCMR and TAMR in southern Belize. To properly scale up reef replenishment in each targeted MPA, equipment and trained crews should be established in each MPA, and mapping of extant corals must continue nationwide. Another two-three years (at least) focused in these two MPAs would allow for the advanced genetics analysis to be applied, and eventually the diver-based photo-mosaic results will allow calculations of ideal outplant density (fragments/m^2); in theory this will allow for fewer outplants in more areas, instead of concentrating many outplants in few areas, and allow further evaluation of the different growth rates across Belize.

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