INCEPTION REPORT v 1.2

Repopulate Reefs within Replenishment Zones of Turneffe Atoll Marine Reserve and South Water Caye Marine Reserve with Temperature Resilient Coral Varieties

MCCAP
BELIZE MARINE CONSERVATION & CLIMATE ADAPTATION PROJECT

ADAPTATION FUND

WORLD BANK GROUP

Fragments of Hope

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1.0 Executive Summary/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 Forestry, Fisheries and Sustainable Development (MFFSD) 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), 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 Adapation Fund, these activites 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 document outlines in detail the steps necessary to expand the reef repopulation success to Turneffe Atoll and South Water Caye Marine Reserve through 2020.

1.1.1 Introduction

Why focus on the Caribbean acroporids?

The Caribbean acroporids were the first corals to be listed on the IUCN Red List (Aronson *et al*. 2008) and prior to that were listed in the U.S. as Endangered Species (NMFS 2006). Their population decline has been documented and assessed at over 95% loss in recent decades, directly attributed to climate change effects such as increased frequency and intensity of storms, bleaching and disease event associated with elevated sea temperatures (Aronson and Precht 2001; Bruckner 2003). The Caribbean acroporids are considered keystone or foundation species in their ecosystem because they are the main reef building, branching and previously dominate species in shallow to mid-depth ranges, providing shoreline protection and critical habitat for hundreds of other marine species (Precht *et al.* 2002, Bruckner 2003). A recent study found that shallow (live) reefs dissipate as much as 86% of wave energy (Beck *et al.* 2014) and due to many dead reefs now turning to rubble, emphasis of rugosity (structual complexity of reefs) for providing fish habitat has become adopted in montoring protocols (AGRRA[1]) and management programs (Graham and Nash 2013). Because the Caribbean acroporids are relatively fast growning and easy to propagate, Critically Endangered and provide valued ecosystem services, they are the logical first choice for reef repopulation/restoration efforts (Lirman *et al* 2010*;* Johnson *et al*. 2011; Young *et al.* 2012; Lirman *et al.* 2014; Mercado-Molina *et al.* 2015). The Caribbean acroporids are considered so essential and irreplaceble for functional reef ecosystems that NOAA[2] has developed a Species Recovery Plan (NMFS 2015) and are currently working to expand the efforts regionally, outside of U.S. territories, and as such, FoH is partnering with their team(s) for future regional workshops and collaborations.

1.1.2 History of Fragments of Hope

Fragments of Hope (FoH) was registered in Belize as a not-for-profit organization in September 2013. FoH has a five member board, all born Belizean Citizens from the Placencia peninsula community. Lisa Carne, a naturalized Belizean, is the Founder and Executive Director, residing in Placencia over 20 years, and general membership exceeds 30. General membership meetings

[1] http://www.agrra.org
[2] http://www.coris.noaa.gov/activities/elkhorn_recovery_plan/

are held annually, with board elections every three years. While FoH was founded with reef replenishment as its priority, the founder and members also have extensive experience with other marine ecosystem monitoring skills, capacity building and educational outreach activities. These include past successful collaborations with the Placencia Tour Guide Association (PTGA) for two US$50,000.00 (each) projects for capacity building and marine monitoring, and two grants (US$55,000) with the Placencia Producers Cooperative Society, Limited (PPCSL) for farming seaweed. Current projects (US$84,000) also include mangrove reforestation and lagoon ecology training for licnesed tour guides. For this reason the FoH mission statement reads thusly: *Fragments of Hope, Ltd (FoH) is a non-profit, non-governmental, membership organization dedicated to promoting and implementing active management solutions for threats to the Belize Barrier Reefs Reserve System and related ecosystems*. More information on the organization can be found at the website fragmentsofhope.org under the specific tabs, Programs:About Us and Donate: Membership program.

Since formal registration in Belize, FoH has successfully completed three short-term consultancies for the World Wildlife Fund-Central America (WWF-CA) under the title, "Facilitating coral reef resilience to climate-driven bleaching incidence thorugh bioengineering as a means of lesson-learning: A continuation", totalling US$52,449.00. FoH has also recently completed an 18-month contract for hire with the InterAmerican Development Bank (IDB) entitled "Coral Reef Restoration Program (RG-T2381)-Applied Adaptation" for US$230,000.00. These larger programs and funds, accessible only as a registered organization, allowed the successful scaling up of reef repopulation efforts in southern Belize and necessary scientific collaborations begun by FoH Founder Lisa Carne with smaller research grants 2006-2013 (funded by PACT, Project AWARE, WWF-CA, CCCCC, WB). FoH is also currently finalizing[3] combined grants (US$84,000) from COMPACT and CARIB SAVE for the project entitled "Enhancing mangrove and coral ecosystems via active reforestation/restoration efforts and structured training activities in the Stann Creek District".

[3] All deliverables have been met with the exception of continued mangrove reforestation monitoring, which will be complete in the next two months, and therefore not conflict with the MCCAP inception.

Lisa Carne and now Fragments of Hope have pioneered reef restoration in Belize (since 2006) and is the only organization in Belize with approval from the government for reef replenishment work. As such FoH has developed a three-day training curricula (with manual and training videos) vetted by the Belize Fisheries Department and held the first Reef Replenishment training workhop in January 2016 (funded by COMPACT and CARIB SAVE) with 18 successful participants. This manual is shared in Annex 6.2. and this training program will be repeated annually under the MCCAP consultancy. FoH has an excellent working relationship with the Belize Fisheries and Forestry Department, the University of Belize, marine conservation NGO's, as well as vast experience working with coastal community stakeholders and an extensive network of regional and international scientific collaborators.

1.1.3 Results of reef population work to date in Belize

Mapping acroporids began in Belize in 2006, and while the work has focused in southern Belize, extensive data has already been collected by FoH in other areas, and data from AGRRA surveys UB and SI surveys are also accessible by FoH. See section 1.3.1 for existing maps.

Genetics on acroporid hosts has been collected since 2007, and combined with symbiont genetics since 2009. In 2007, the donor reef for the first *A. palmata* transplants at LBCNP was surveyed using a concentric circle pattern (Baums *et al.* 2005) and found to have a genetic diversity of 0.7. This means that of 24 corals sampled, 17 were unique genets or different individuals of the same species (17/24). Knowing the natural genetic diversity is important for restoration goals of any species, to mimic natural diversity, and in the NOAA Species Recovery Plan for Caribbean acroporids (2015) they list a target genetic diversity 0.5 for both *A. palmata* and *A. cervicornis*. Less information is know about *A. cervicornis* natural stand diversity, with assumptionins thpast that large stand are typically monotypic (one genet that spreads by asexual propagation). More information on this will be available in the course of this consultancy, as within stand genetics were sampled from natural patches in southern Belize by D. Lirman and FoH is awaiting results; there are also pending resutls from other reserachs on *A. cervicornis* in northern Belize and Florida.

In general, more genetic diversity is better than less, with an important caveat, a concept adopted from terrestrial genetics known as "ecotypes". In terrestrial systems it can be trees of the same species adapted to different elevations on a mountain, amount of sunlight or rainfall, for example. For corals (holobionts, including the symbionts and microbiome community), it can mean some individuals of the same species are specially adapted for different depths, temperatures, wave energy, light exposure, etc. While there are no 'rules' as yet, in general corals would not be transplanted great distances from their source or to differetn micro-habitats, *except for experimental purposes*. This is why the scoping/mapping/ genetics is a crucial component to reef replenishment, and to date the focus ahs been in southern Belize.

Outside of the donor reef assessment in 2007, 40 acroporids from southern Belize have had their host genetics analyzed (Baums *et al* 2009 and 2015, Fig. 1a) and over 90 acroporids have had their symbiont genetics analysed (Fig. 1b-c). Regarding the symbiont results, all of the A. cervicornis that harbored D1a in 2009-2010 were reun in 2015 and found to now harbor symbiont A3.

There are 19 table nurseries already established in southern Belize, and three remaining A-frame[4] nurseries from 2009 (Fig. 2) that hold starter fragments from the acroproids mapped and with known genetics. Recent additional nurseries were added in the replenishment zone in Gladden Spit and the Silk Cayes Marine Reserve (GSSCMR), and near Moho Caye as a control, since Moho caye is unprotected waters. Some of these nurseries have been completley harvested and so may be removed; the rest have had their corals trimmed sufficiently to minimize maintenance.

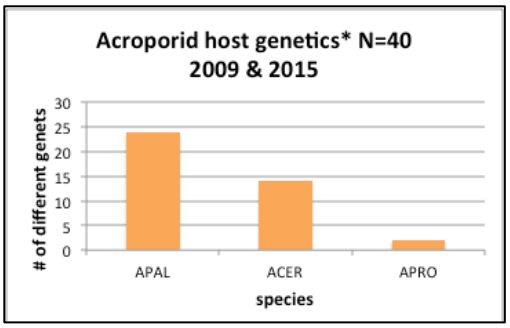

Fig. 1a. Acroporid host genetics completed to ensure multiple individuals of each species are included.

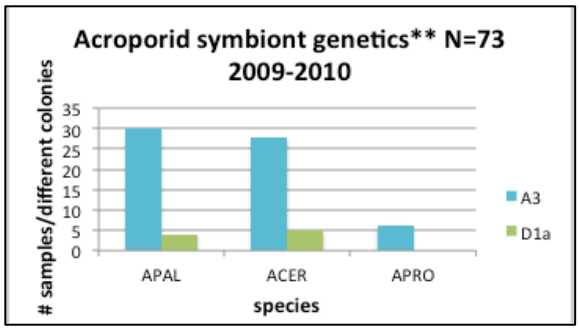

Fig. 1b. Results of symbiont gentics for 73 acroporids through out Belize main barrier reef, 2009-2010.

[4] This nursery method has been discontinued for several reasons, including longevity of the material and overcrowding of corals on the A-frame.

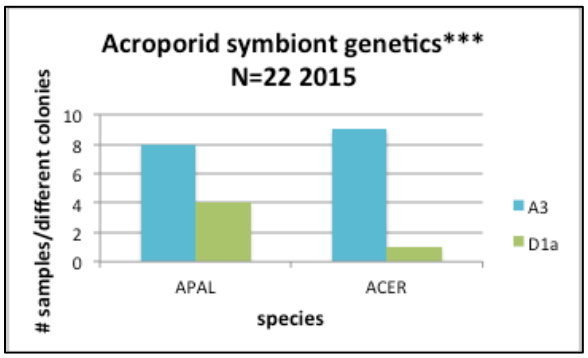

Fig. 1c. Additional symbiont genetics were rerun in 2015 when it was found that all A. cervicornis that harbored D1a in 2009-2010 now harbor symbiont A3.

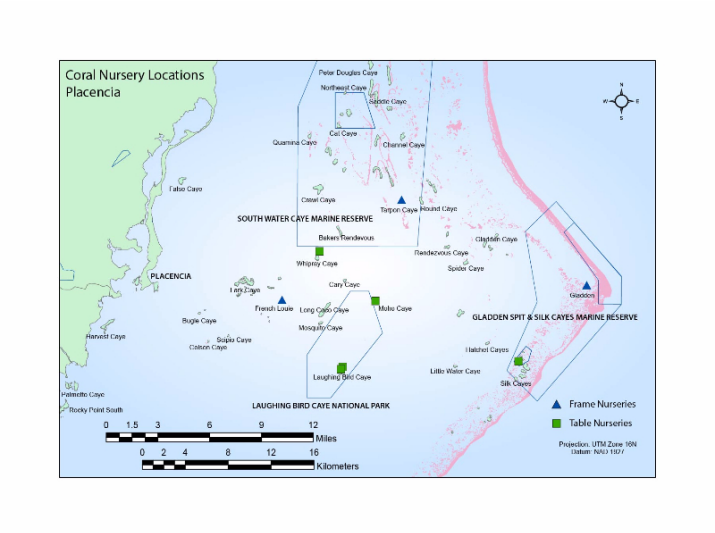

Fig. 2. Map of nursery locations and types, near Placencia. There are 19 table nurseries and three frame nurseries. Although the frame nurseries will no longer be installed, the remaining ones continue to be harvested.

Monitoring of nurseries includes growth and survival rates, given known host and symbiont genetics. A recent example of several *A. cervicornis* gentotypes in multiple nursery locations is shown in Fig 3. Methods are fully described in section 2.2, this is just one example of results to date. Other routine monitoring of nurseries includes bleaching and disease and *in situ* temperature, one example of temperature given in Fig. 4.

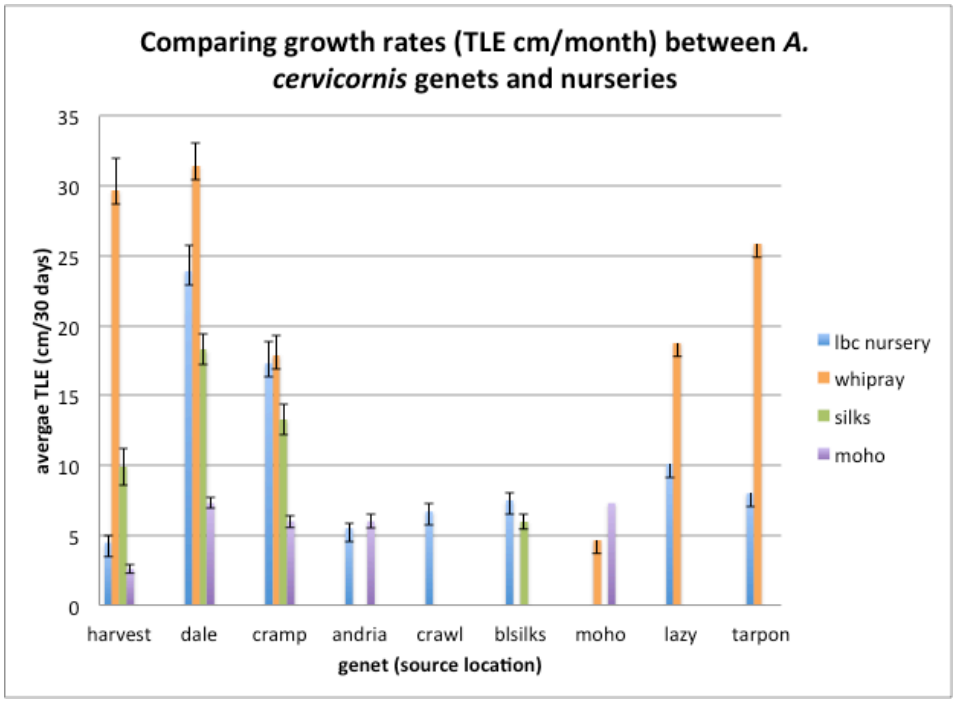

Fig. 3. Comparing growth rates between nine *A. cervicornis* genets (X-axis) in four different nursery locations (color coded). TLE=Total Linear Extension averaged out for 30 days (cm/month). Total growth days measured varied between 56-208 days. Error bars are SE for each data set and will be updated.

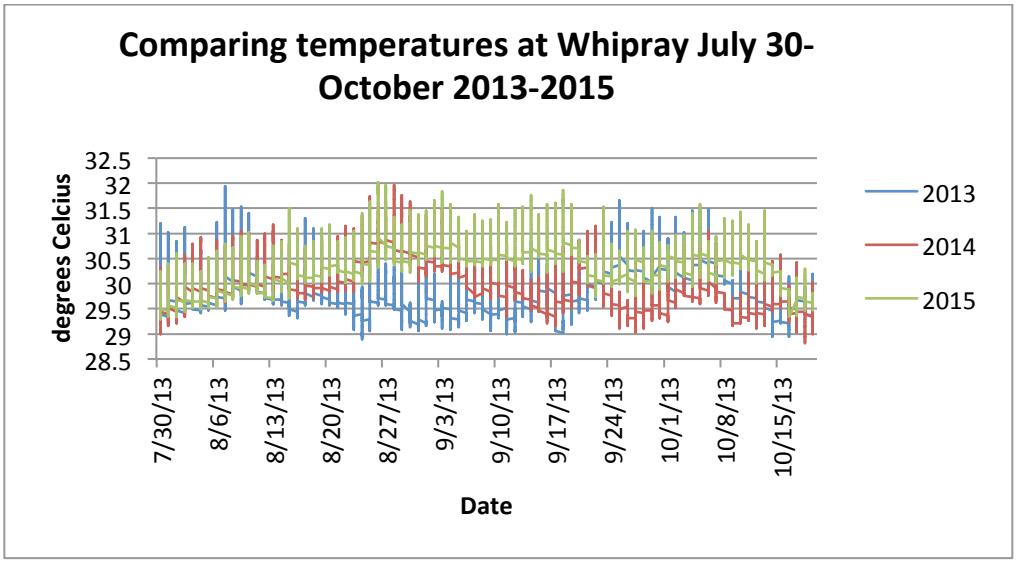

Fig. 4. Comparing *in-situ* temperatures (degrees Celsius) at Whipray Caye between 30 July and 20 October across three years: 2013 though 2015. The data indicate higher temperatures by a whole degree in August –Sept 2014 (red lines) vs. 2013 (blue lines) and by two degrees in 2015 (vs. 2013)

Repopulation results from southern Belize:

Over 59,000 nursery-grown acroporids have been outplanted to LBCNP to date, with an additional 11,507 fragments recently added to Moho Caye (as control, outside of protected areas) and another 5,680 fragments at S. Silk Caye this year. Figure 5 is a pie chart illustrating the

amount of corals outlanted at LBCNP only, 2006-2016. Over half of the outplants occurred in 2016, which is reflection of the amount of nurseries scaled up since 2014, adequate funding, appropriate weather conditions and significantly, the teams' increased proficiency and efficiency.

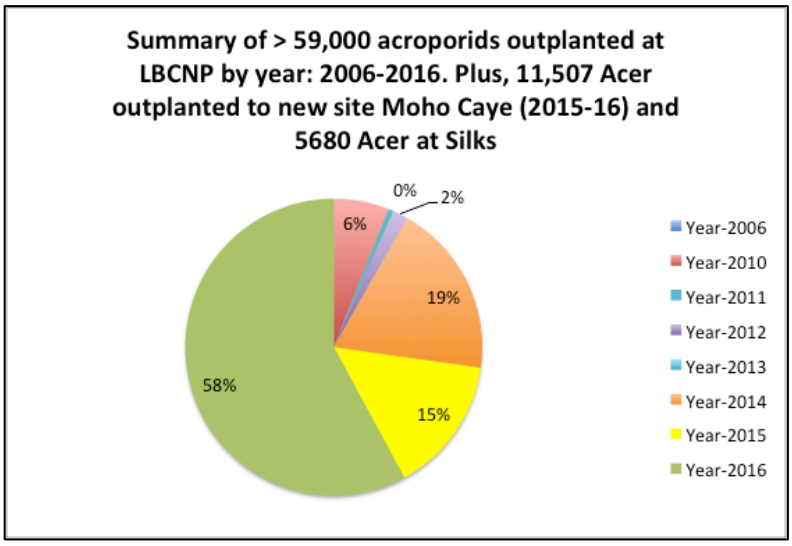

Fig. 5. Pie chart illustrating number of corals outplanted by year; over half of over 59,000 corals were outplanted in 2016, a reflection of multiple nurseries established and appropriate funding.

Survivorship and growth rates of acroporid outplants are difficult to monitor and quantify, because of their natural abilty to asexually regenerate and fast three-dimensional growth. This is a justification for the recent use of photo-mosaics to quantify increased coral cover over time (next section). Because *A. palmata* grow slower than *A. cervicornis* and their morphology as a single 'colony' is easier to assess than the branching *A. cervicornis*, and example is given in Fig. 6a-b, on the original *A. palmata* survivorship from the first tranplants in 2006, and the nursery grown *A. palmatas* outplanted since 2010, at LBCNP. *A. plamata*, like all acroporids, can break naturally in storms, or from their own weight, or from from fin damage, creating asexual replicates or fragments from the original colony termed 'satellite colonies'. Sometimes they settle naturally with their weight and grow where they land, and sometimes they reaffixed with cable tie or other methods. In the simplest example, 19 *A. palmata* fragments were transferred to LBCNP in 2006, one died in the first year (was dislodged and not rescued in time), one was added in 2007, and one died (unknown causes in 2010). So simple survival rates in 2016, ten years later, could be calculated as 90%. But as Fig. 6a illustrates, this is not the true picture, since a recent count (May 2016) revealed a total of 48 discreet coral colonies from the surviving

original 18 *A. palmata* transplants. Similarly, the 187 nursery-grown outplanted *A. palmata* since 2010 now number 234, with only one loss (dislodged). Because these survivorship and growth rates are so difficult to measure, especially for *A. cervcornis* and *A. proliferaI,* FoH has taken a more holistic approach to measure the percent live coral over increased in sub-plots within sub-sites at LBCNP. Using these methods (detailed in section 2.3), live acroproid cover has increased from zero to over 35% at LBCNP since 2010 (Fig. 7a-b). Fig. 7a illustrates two years of photo mosaic data (2014 orange bars and 2015 green bars), with the unplanted (2014) sites (UP1 and 2) on the left, sites 20 and 21 were outplanted just five months before the 2014 mosaics, and sites 9 and 13 were outplanted in late 2010. Although the bars represent total live coral cover, the change/increase in 2015 (green bars) is all due to rapid acroporid growth in just one year. Fig. 7b shows the breakdown in live coral cover by species, and represents less than five years' growth of the outplanted acroporids: from a baseline of zero acroporids in 2010, the three acroporid species had over 35% coverage at site 13 in August 2015. Without the replenishment/repopulation work, this site would have less than 5% live coral cover.

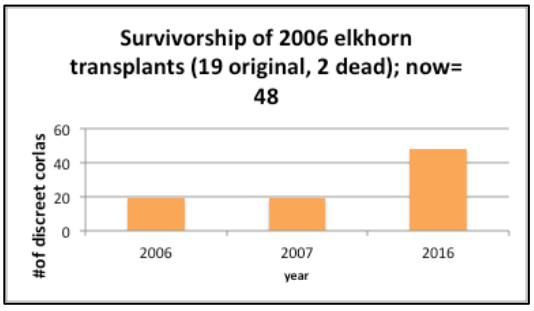

Fig. 6a. Illustrating the survival of the first 19 elkhorn transplants at LBCNP. One was lost and one was added in 2007, and another died in 2010, making the survival rate almost 90% in almost ten years. But because of the multiple satellite colonies created over this time, this simple math is not a true reflection of the survival rate (2016 count done 27 May 2016).

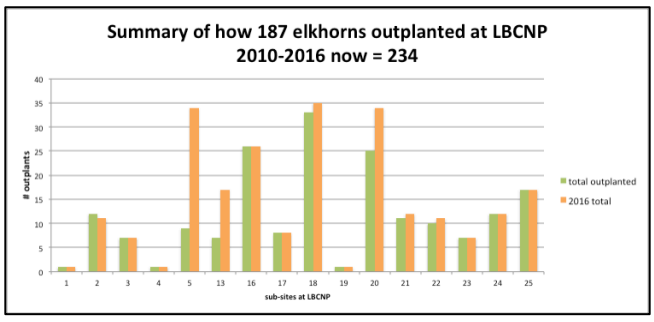

Fig. 6b. Illustration of the exact numbers of elkhorn corals outplanted at each sub-site in LBCNP 2010-2016 (green bars); one 'lost' at site 2, but others increased by fragmentation at sites 5, 13, 18, 21, and 22 (orange bars, counted 8 June 2016). Simple math gives a survivor rate of 99%, but again does not reflect the true coverage of corals.

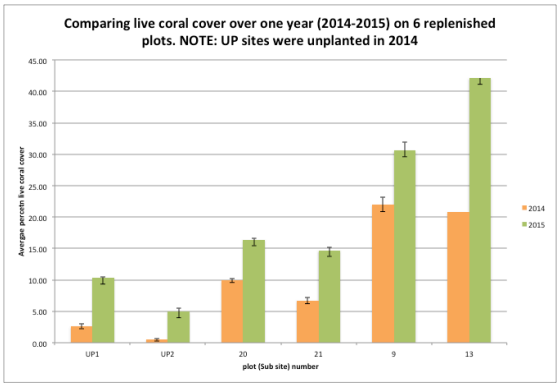

Fig. 7a. Comparing live coral cover on six replenished plots using mosaics analyzed with CPCe software. The error bars represent standard error. The UP sites were unplanted in 2014, all other sites had been previously out-planted.

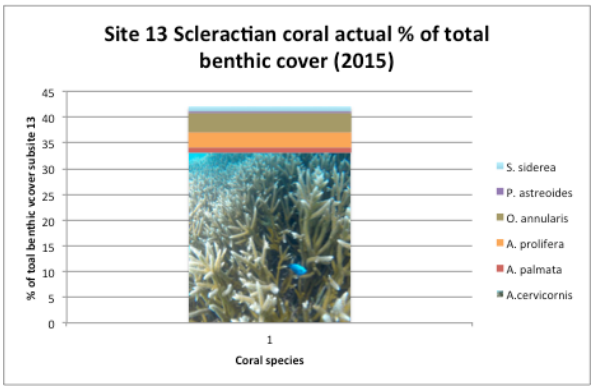

Fig. 7b. Accurate stacked graph shows percent coral cover by species, relative to total benthic cover; site 13, 2015

Figures 6a and 7a illustrate well how acroporids can self-regenerate and spread their cover with asexual fragmentation and fast growth, but corals are animals and can also sexually reproduce. Although they are hermaphrodites (not all coral species are), they cannot self-fertilize, thus another justification for the genetic analyses is to ensure different individuals of the same species are outlanted in proximity to each other to allow for fertilization after spawning events. All three nursery grown acroporid species were documented spawning[5] at LBCNP in 2015; there is also evidence (histology slides of gamete formation) to suggest the *A. cervicornis* were spawning at LBCNP less than two years after being out-planted.

1.1.4 Synergies with past repopulation work (2006-2016)

The mapping and genetics analyses planned under this consultancy complement the work already undertaken since 2006-present, and are a continuation of efforts in Belize to identify resilient reefs, and ensure that the Critcially Endangered acroporids never cross the line to "Extinct in the Wild' on the IUCN Red List, as has already happened in Barbados, for example.

1.1.5 Synergies with overall MCCAP objectives & MPA management plans

The overall MCCAP objective is to combine ecosystem based adaptation with policy strengethening/changes and expansion of the Replenishment Zones. Reef repopulation efforts

[5] A short clip of the 2015 nursery-reared coral spawning event is shown in the video on the home page of fragmentsofhope.org.

directly meet the first part of the goal, ecosystem based adaptation, by identifying thermally tolerant acroporid genets and increasing their abundance in strategically chosen sites, ensuring multiple inviduals are placed in proximity to sexually reproduce, and using genetics and long term monitoring to track their progress and document associated increased biodiversity with restoring these keystone species.

Both management plans for SWCMR and TAMR identify climate change as a very high threat to the coral reef ecosystem, and list percent cover of live corals as a conservation target and monitoring indicator. While the management plans do not specifically mention repopulation/replenishment efforts as a conservation strategy, regionally and globally this is an accepted and now embraced management tool. When this consultancy is successfully completed, and based on the documented success to date at LBCNP, it is likely that reef repopulation will be included in updated and future MPA management plans.

1.2 Outline of Approach for this consultancy

The approach for reef repopulation in SWCRM and TAMR follows the successful approach used in southern Belize to date, with a few new technical additions (e.g. the larvae dispersal map), and follows the same order described in the Methodology Section 2.0:

- Coastal stakeholder consultations; in the beginning of the consultancy to raise awareness, collect anecdotal information on existing acroporid stands in each MPA from fishers and tour guides, and recruit participants/trainees for the work, and at the end to share results on the reef repopulation results, and specifically nursery and out-plant locations in each MPA.
- Mapping: using existing data sets, collected anecdotal information, remote sensing (when/where possible), and ground-truthing.
- Genetics analyses: to ensure diversity of nursery reared and out-planted acroporids, and to contribute to ongoing research on the role of the symbiont versus the host for overall resilience.
- Rapid reproduction of corals using established *in situ* nursery methods (and inclusive of regular monitoring such as temperature, growth rates, survival, bleaching and disease resilience).

- Out-planting of nursery-grown coral using established methods.
- Documenting longevity, increased coral cover, and related increased biodiversity using established methods.
- Sharing results not only with the coastal community stakeholders, but also with the local, regional and international scientific and reef management and policy makers' communities.

1.3 Scoping and Consultancy Activities to Date

1.3.1 Existing Maps

Figure 8a is the most recent map of the legally designated replenishment zones in Belize (provided by TNC), but FoH will work closely with the Fisheries Department to be informed of recent consultancies for expansion or addition of any replenishment zones in both SWCMR and TAMR. Figure 8b is a map of *A. cervicornis* distribution made from ground-turthing, and Fig 8c illustrates the added info from historical AGRRA surveys. These maps are from 2010, and will be updated under this consultancy, and exist for all three acroproid species.

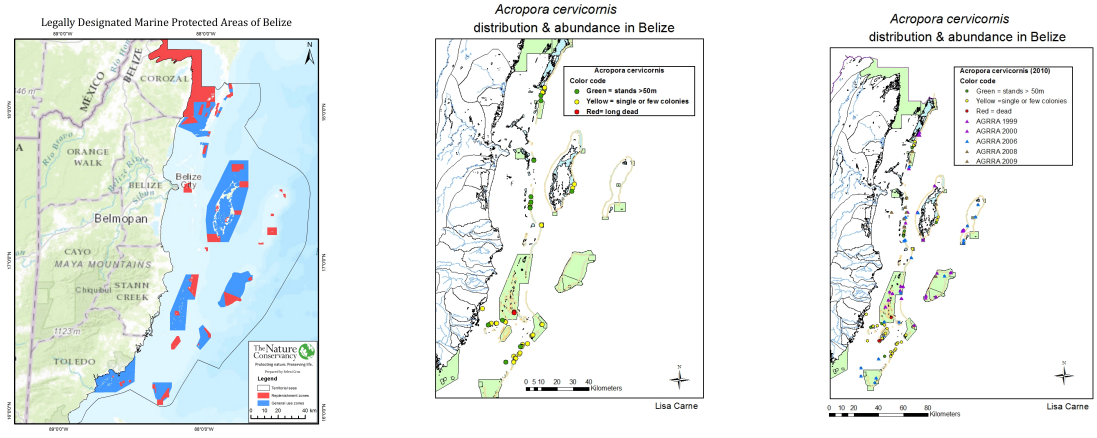

Fig. 8a-c. Current map of replenishment zones in Belize; 2010 map of *A. cervicornis* distribution in Belize; 2010 map of *A. cervicornis* distribution in Belize including historical AGRRA data.

Figures 9a-b are examples of maps created without GIS software: Fig. 9a is an example of *A. cervicornis* locations near Placencia created with Google Earth, Fig. 9b is a map of outplant

locations around LBCNP created in Adobe Photoshop, color coded for years out-planted. Fig. 9c is an accurate map of outplants at LBCNP with actual GPS coordinates, but not updated. While there do exist older maps of wind and current patterns for Belize, these will also be updated under the MCCAP consultancy, when the larvae distribution map(s) are generated.

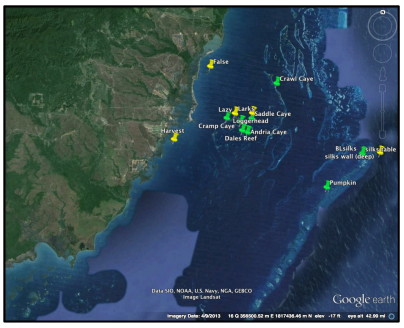

Fig. 9a. Map of *A. cervicornis* distribution near Placencia made with Google Earth.

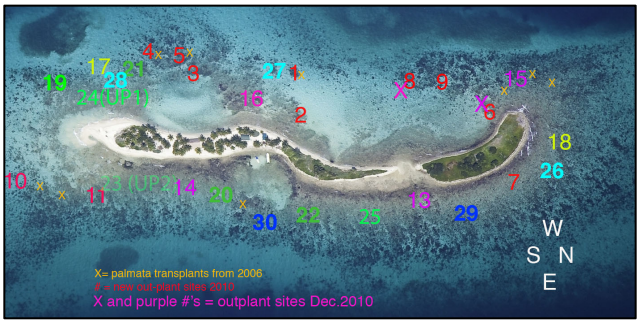

Fig. 9b. Handmade map of outplants sites at LBCNP color-coded for the years outplanted.

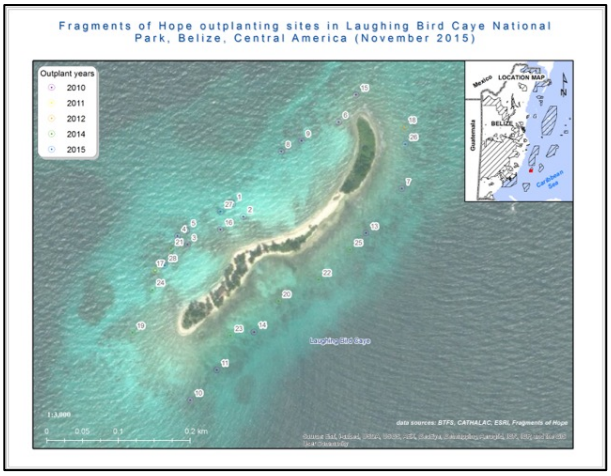

Fig. 9c. Accurate map of LBCNP outplant sites using actual GPS coordinates (but not updated since 2015).

1.3.2 Review of Stakeholder consultancies 2014-2016

In the past two years only, three public consultations have been held in Placencia Village; the most recent occurred 14 June 2016 with over 60 attendees, highlighting increased awareness and interest on coral reef health and recovery. Consultations were also held in Belize City, San Pedro, Dangriga and Hopkins Village. Belize City is a listed community for TAMR, and Dangriga and Hopkins are stakeholder communities for SWCMR. The Belize City consult was mostly government and NGO representatives, but both Dangriga and Hopkins were primarily fishers and tour guides. At all consults a specific topic (besides dissemination of the reef

replenishment work to date) discussed was the use of foreign volunteers in reef replenishment work, since there are regular requests from non-Belizeans to participate in the work. The conclusion was to restrict the "hands-on" work (nursery and outplanting) to trained coastal communities stakeholders for several reasons: even volunteers need a work permit in Belize, a special research permit is required to handle coral, training takes a minimum of three days plus previous knowledge of Belize's marine species and ecosystems and advanced comfort/experience level working underwater, because many of the trained guides and fishers receive a small daily stipend equivalent to tourism pay and it is hoped these skills will one day be recognized as an alternative livelihood income source, and most importantly, trained participants gain renewed pride and appreciation and a sense of ownership for their coral reefs.

At both the Dangriga (July 2014) and Hopkins (October 2014) consults, mention was made of the pending MCCAP consultancy to expand the work in SWCMR, and particpants were unanimous in their support and eager to begin the work in their areas.

2.0 METHODOLOGY

The methods follow the same order as the approach: mapping (with stakeholder consultancies included), genetics (host and symbiont) analyses, establishing nurseries, monitoring of nurseries (growth and survival rates in conjunction with genetic information), out planting of nursery reared corals, monitoring the repopulated reefs for coral cover increased, survival, and associated biodiversity.

2.1 Mapping (Task 2): Identification of reefs suitable for nurseries set-up and out-planting

Mapping of extant acroporid reefs is a crucial first step in planning for new restoration sites, and also contributes to the national research agenda item, 'identification of resilient reefs". While mapping has ensued since 2006, Belize has the second longest barrier reef in the world, and three extensive atolls. Mapping of TAMR and SWCMR can begin with stakeholder community consultations to garner anecdotal information from fishers and tour guides. The use of satellite

images and existing AGRRA[6] data as well as previous studies (e.g. UB has done some acroporid mapping in TAMR, and the Smithsonian Institute has also done some detailed mapping near Carrie Bow in SWCMR; Boston University has also has some data from both MPA's and FoH has this data) tells at least some presence/absence of acroporids. "Ground truthing" involves actually visiting each site but requires only snorkeling (due to the acroporids natural shallow depth distribution), use of cameras, and GPS. The equipment will be provided and owned by Fragments of Hope. The sites are then assessed with the following three categories: long dead stands (red), recovering or remnant colonies (yellow), and stands in excess of 25-50m (green). An example of this type of map made with GIS software is shared in Figure 8b-c[7]. Fig. 8b illustrates the mapping conducted as of 2010 (needs updating) with the necessary parameters for nursery work (size of stands); Fig. 8c shows the additional data set from previous AGRRA surveys that only reveal presence of the species-this is the starting point for ground-truthing activities. Besides contributing to the abundance and distribution of these Critically Endangered species, the bigger picture includes a computer spawn simulation or larvae dispersal map (outside consultant Claire Paris from University of Miami). Figure 9a shows a map of *A. cervicornis* generated using Google Earth, free software. Color codes indicate the size of the stands (large=green) or single to few colonies (yellow).

This computer spawn simulation can be created with existing knowledge of wind and current patterns (existing), benthic habitat data (eg. sand, reef, sea grass, some exists, some will need to be ground truthed), days until larvae settle (days until competency) and preferred habitat (known), and acroporid population data (to be generated by the above mapping techniques). The purpose of this dispersal map is to give the best data-driven estimates of where acroporids are already naturally reseeding reefs, and then fill in the gaps with new nursery/restoration sites, and combine that with existing site selection criteria. This is the first and only known national restoration plan for acroporids that takes larvae dispersal into account, and is fully endorsed by the Belize Fisheries Department.

At the same time field mapping is done for the corals to include in nurseries, genetic samples are

[6] http://www.agrra.org
[7] These maps (inclusive of the GIS software training) were funded by WWF 2010-2011 and need to be updated.

collected. Genetics analyses of both host and clade only requires ~ 1cm of coral, which are stored in pre-made vials with 70-90% ethanol supplied by Baums' laboratory at Penn State[8]. Methods are in papers Baums *et al*. (2005, 2009). Corals are assessed for host genetic diversity and symbiont algae clade type.

Nursery site and out-plant location selection will also occur via community consultations and during the physical mapping. Below is a short list of site selection criteria for nurseries and outplants. The completed manual for the training course is provided as an Annex (6.2) and outlines each of the activities under Methodology.

Nursery site selection criteria:

- Accessibility (fuel considerations)
- Optimal depth 2-5m
- Clear, good water quality and flow (presence of healthy corals)
- Protection from high surge (leeward side of cayes, nestled amongst large coral heads)
- Sand and/or rubble substrate or sparse seagrass and sand (test with probe and mallet)
- Permanent residents on caye or nearby
- MPA status/protection
- Permission /endorsement from managers/co-managers if in MPA
- Stakeholder support

Proximity to out-plant site and cross reference with out-plant site selection criteria

Outplant site selection criteria:

- Accessibility (logistics for long-term monitoring)
- Evidence of acroporids (dead and alive)
- Clear, good water quality and flow (presence of healthy corals)
- Low macro-algae cover
- Crustose coralline cover
- Presence of *Diadema antillarum*
- Presence of parrotfish/surgeon fish
- Solid/fixed substrate (not rubble-can use domes on rubble)
- No-Take (replenishment) zone status)

After the larvae dispersal maps are completed, these will be used with the above criteria, for strategically located replenished outplant sites to promote natural reef regeneration.

[8] http://www.personal.psu.edu/ibb3/Iliana_Baums.htm

2.2 Establishment of coral nurseries in TASA and SWCMR (Task 3) inclusive of monitoring

The nursery tables are ~10 x 10 feet made with rebar steel (5/8th inch) and support the rope and 'cookie' culture (for *A. cervicornis* and *A. prolifera*) as described in Bowden-Kerby and Carne (2012)[9], and shown in Figures 10a-b. Further detailed descriptions below.

Cookie-tray Culture

Cookie tray culture consists of massive and small branched corals being planted onto 10-15cm cement disks, each held in place by fishing line woven through the four holes in each "cookie". The cement cookies or buttons are attached to a wire mesh tray made of 1cm x 1cm plastic coated mesh, with the cookies woven through like giant buttons with 80 pound-test fishing line, so that the line crosses diagonally from the holes, forming an X on top of each cookie. Corals are woven into this array, held onto the cookie by the fishing line X. The completed trays are placed on and attached to a metal table constructed with 5/8 inch metal rebar.

Rope Culture

Rope culture consists of twisting ¼ inch poly rope so that a hole opens up between the three major strands, and simply inserting a small (5-15 cm) coral branch into the opening, and then releasing the twist so that the rope closes down on the coral, holding it in place. The ropes are suspended ~1 meter above the sand or seagrass substratum tied between two 5/8 metal bars attached to the coral table with the cookie trays attached.

Each table can support up to ten (10) ropes with 10-12 fragments each. Six tables would support up to 720 starter fragments of *A. cervicornis* and/or *A. prolifera*. These fast growing species can be harvested at 7-13 months, with each rope yielding ~ 100 fragments. Six tables can potentially yield a maximum of 6,000 fragments for out-planting, annually.

Each table can also support 48-120 'cookies' for *A. palmata* (and other stony coral species), which need ~12-14 months in the nurseries, translating to between 288-720 *A. palmata* out-

[9] Bowden-Kerby, A. and Carne, L. (2012) Thermal tolerance as a factor in Caribbean *Acropora* restoration. *Proceedings of the 12th International Coral Reef Symposium*, Cairns, Australia, 9-13 July 2012 20A Restoration of coral reefs.

plants.

Mother colonies will include the acroporids with their coral host and symbiotic clade previously typed (Task 2) from Turneffe and SWCMR. Incorporating a high level of genetic diversity into restoration efforts is vital (Baums 2008, Shearer et al. 2009, Baums et al. 2010). Genetic work on coral allelic diversity (Shearer et al. 2009) has indicated that ten randomly collected parent genotypes will preserve >50% of the genetic diversity within a coral species. However, the study indicates that it requires 35 genotypes to obtain >90% of the original genetic diversity.

Fig 10a. Example of table nursery showing 'cookie' and rope culture.

Fig. 10b. Example of table nursery at six months.

Monthly monitoring of nurseries is important and especially after weather events. It is crucial in warm water months for removal of fast growing alga species, and to record bleaching/disease events, and/or mortality.

Photography is an essential monitoring tool; the monitoring budget includes several basic underwater cameras for monitoring for each site/location/MPA, and one professional underwater camera for macro- and night photography needs. Thousands of monitoring photos and videos will be generated; a large storage capacity laptop and multiple external hard drives are included in the monitoring budget for each location. Organizing (labeling and editing) photos and videos are essential and time-consuming monitoring and record-keeping needs.

Monitoring also includes survival and growth rates in the nurseries. Growth rates for *A. cervicornis* on ropes will be collected using the Total Linear Extension (TLE) protocol described in Kiel *et al*. 2012. Ropes are assembled with 9-15 replicates, and measurements taken in cm

Day 0. At subsequent data collections each apical branch is measured and totaled, then the Day 0 value is subtracted. The value is divided by total growth days measured (variable for each genet/location) and multiplied by 30 days for growth in cm/month. Each replicate value is averaged. If growth rates are taken for any other species they will use the AGRRA method (three dimensions for colony volume).

2.3 Out-planting of selected reefs (Task 4) inclusive of monitoring

Three out-planting methodologies include using cement (Fig. 12a), pegging (nailing) ropes into substrate (Fig. 12b) and tightly wedging fragments into crevices in dead reefs, not shown (Bowden-Kerby and Carne 2012). Corals are harvested from the nurseries and transported in large containers, with seawater flushing them constantly, to the out-plant sub-site (Fig.11a-b). Current limitations are the size of the containers, especially for the larger *A. palmata* cookies and the size of the vessels to transport corals. The containers shown in the figures can hold ~250 *A. cervicornis* fragments; it may take a single diver ~ 30 minutes to harvest this amount of corals, and ~90 minutes to out-plant them. Ideal weather and sea conditions are overcast, cool and calm waters, both for the transporting and the cement work. Corals can only be harvested and out-planted outside of the hurricane season (December-May) to minimize stress and maximize survival rates. This coincides with the area's high tourist season, and since traditional assistants have mostly been tour guides who are often busy this time of year, there is a travel category included in the out-planting budget to bring qualified assistants (Fisheries and NGO and/or university staff) from other districts in Belize to Turneffe and SWCMR as necessary. These will include past participants from out-planting workshops and/or new trainees. A minimum of 500 corals with one team/day can be out-planted. Therefore a target of 10,000-20,000 coral out-plants would need single team (of four-five people) 20-40 days total.

Currently the corals planted at each sub-site are recorded into Excel spreadsheets; records include number of each species, mother-location/nursery source, genotype and clade (if known), date planted and planting methodology.

Fig. 11a. Harvesting corals.

Fig. 11b. Transporting corals.

Fig. 12a. Out-planting with cement.

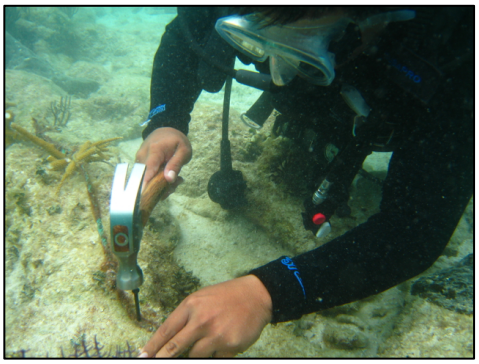

Fig. 12b. Out-planting ropes with nails.

Monitoring of out plants includes documenting survival and bleaching and/or disease, similar to the nurseries, using photos and videos and underwater slates. It also includes documenting and removing, if necessary, coral predators such as fireworks and snails. After a certain age and size, growth rates are difficult to measure on acroporid colonies, due to their multi-branching morphology and three-dimensional growth. FoH instead has begun using a photo mosaic protocol, that allows us to evaluate the percent of live coral cover increased over time, in specific plots (50-180m^2). Examples shown in Section 1.1.8, Figs. 7a-b.

The photo-mosaic protocols from Art Gleason at the University of Miami[10]. Three protocols are for SLR cameras (single lens reflex cameras, Nikon D7100), GoPro cameras, and for video in general versus still frames. The different camera models/brands reflect the difference in high (Nikon) versus low (GoPro) resolution. These protocols describe the cameras' settings and diver swim patterns. Modifications include physically measuring the plots sizes by installing semi-permanent corner markers and using transect tape for perimeters and diagonals lengths for exact

[10] http://yyy.rsmas.miami.edu/groups/reidlab/

plot area calculations. The cameras are placed on a length of PVC pipe with their housings screwed into place. Two levels are placed on the PVC pipe as well as added Styrofoam floats to adjust for buoyancy. This set up allows one swimmer to handle all the cameras at once.

The methodology to analyze the completed photo-mosaics for live coral coverage from the photo-mosaics jpeg files is CPCe, "Coral Point Count with Exel extensions"[11] a free software from Nova Southeastern University. CPCe gives information on other benthic phyla/species as well as the targeted acroporids.

Biodiversity can be measured with photos for species identification and cataloging and/or with fish surveys. The following protocol for assessing fish abundance and diversity, to compliment the mosaic plots for coral coverage, was developed by Dr. Kaufman at Boston University. Because the mosaic plots are by design, small (50-180m^2), traditional fish survey methods such as AGRRA[12] are not appropriate (minimum of eight 30m belt transects per site). The following methods were used:

A: For individual plots (they were about 10m x 7m):

1- Note all fishes 20cm and greater, and their sizes in 5cm increments, within an area extending 4m outward from the outside edges of the box.
2- Note all fishes less than 20cm and their sizes in 5 cm increments, within a band extending 1m *inwards* from the edges of the box.
3- Note all species not previously observed (in 1 and 2) in wandering over the area first surveyed in 1.

B: For the region in which several plots may be located, lay a 30m transect line out across the area that includes the plots, at the same approximate depth:

1- Note all fishes 20cm and greater and their sizes in 5 cm increments, within an area extending 4m to one side of the transect line.
2- Note all fishes less than 20cm, and their sizes in 5 cm increments, within a band extending 1m to that same side of the line.
3- Note all species not previously observed (in 1 and 2) in wandering over the area first surveyed in 1.

[11] http://www.nova.edu/ocean/cpce/
[12] http://www.agrra.org/method/methodhome.html

Spawning (sexual reproduction) of nursery-grown out-planted corals is a significant success indicator for active population enhancement work. Spawning can be documented visually (which requires night diving and special photography skills). Alternatively fragments can be collected prior to expected spawning times (full moons in July and August), and their skeletons are dissolved to look for presence of gamete formation. Both methods have been used successfully in southern Belize; corals were documented with gamete formation after less than two years on the outplant site, and visually documented spawning after four and five years outplanted. Whether or not spawning is monitored visually will depend on the timing and location (logistics) of the out-planted sites in Turneffe and SWCMR.

3.0. Expected results/Quantifiable indicators

With the exception of the larvae distribution map, a new product/tool under this consultancy, examples of expected results and how they are quantified are shared in Section 1.1.3. Not shared but to be expected, are photos and videos taken regularly as part of the monitoring process, and a large external hard drive will be purchased, to keep a back up copy at the MCCAP office in Belize City. Also not shared in section 1.1.3. are examples of the fish and other biodiversity indicators. Fish biomass data will be shared in graphs for each site, grouped by families and/or functional groups. Other biodiversity indicators may be shared as a species catalogue or list.

3.1 Mapping

Updated abundance and distribution maps for all three acroporid species in SWCMR and TAMR will be created, as well as maps of the nursery and out-plant sites for each MPA. The larvae distribution map will be a deliverable.

3.2. Nurseries

A minimum of six nurseries will be established in each MPA, with data collected on species, mother locations, host and symbiont genetics, growth and survival rates in the nurseries.

3.3 Out-planting

A miminum of three out-planted sites in each MPA, each at a mimium of size of 300m^2. Projected numbers of outplants are at least 6,000 fragments per MPA, with photo mosaics completed on sub-plots of selected sites to measure the amount of coral cover before and after the replenishment work.

3.4 Risks to Implementation

As with all marine related fieldwork, weather is the most crucial factor in meeting deadlines and deliverables. Nursery and out planting work can only occur December 1-May 31, with minor exceptions, because hurricane season is June1-November 30, and also because the warmer water/weather during those months is added stress to the corals. In some years strong winds (outside of actual storms) have delayed fieldwork. Out-planting with cement in shallow reefs requires calm seas; one solution is to out-plant slightly deeper if weather conditions remain poor during the scheduled out-planting sessions. Actual tropical storms and/or hurricanes could also jeopardize the nurseries and/or out-plants. Major bleaching or disease events (sea temperature related) could cause mass mortality. In the worst-case scenario, a no-cost extension may be required if major weather interrupts the work plan detailed below.

Another risk is large changes in fuel costs, as fuel is the bulk of the budget for this work. In the unlikely event that fuel drastically increases beyond the budget, a potential solution is to double up planned activities or 'piggy back' one activity with another.

Finally, another potential risk to implementation is medical emergencies (non-work related) of the principle team members and/or their immediate family. This may be overcome by either allowing a no-cost extension of deliverable dates and/or finding a replacement expert.

4.0 Work Plan

The detailed work plan (by month, by year) is in Table I. During hurricane season (June 1-November 30) no out-planting or adding corals into nurseries is conducted, based on trial and

error in the past (see above section 3.4 Risks to implementation). However regular monitoring, and specifically bleaching and disease montioring, occurs in these months, as well as spawning (typically the August full moon but sometimes also July and September for acroporids). The work plan reflects this below. Consultations and data analyses, report preparations, attendance at regional or international conferences, and contribution to the MCCAP dissemination activities (Task 5.20-22) may also be conducted duing these hurricane season months.

After detailed consultation with Dr. Claire Paris, it was learned she will need a minium of three months solid work, after receiving the last mapping coordinates, to complete her computer models of larvae distribution. Because we have several other site selection criteria in use for choosing nursery and outplant locations, we estimate that one-half to two-thirds of the nurseries may be installed prior to the completion of the larvae distribution map, and the remaining nurseries installed when it is complete. This is also reflected in the work plan below.

4.1 Existing team

The core field work team in Belize comprises five –six experienced members, with another 15 vetted participants from the first Fisheries endorsed training workhshop held in Placencia, January 2016. This training course is schdueld and budgeted for 20 participants, once a year, under the MCCAP consultancy. This three-day traning workshop will most likely be held in December or January of each year. On site training of participating MPA staff and/or slected tour guides and fishers will alos occur as each activity is completed in each MPA.

External team members include Dr. Clair Paris at the University of Miami, a larvae distribution expert, and Dr. Iliana Baums at Penn State, an acroporid genetics expert. Unoffical external team members may include Dr. Art Gleason at the University of Miami for photo-mosaic jpeg creation and analysis.

Table I. Activity (work) Schedule FoH/MCCAP

Deliverables/Tasks/Activities	2016 (YR1)						2017(YR2)											
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Task 1.4 Submit Inception Report	X																	
Task 1.1 Organize briefing with PIAG staff, FD and PACT	X																	
Task 1.2 Submit activities to DOE (env. clearance received, dated 21 Mar16)																		
Task 1.3 Lit review, initial stakeholder consults	X	X	X	X														
Task 2.5a Community consultations SWCMR stakeholders			X	X			X					X						
Task 2.5b Community consultations TAMR stakeholders					X	X							X					
Task 2.6 Training event (3 days)							X											X
Task 2.7a Ground truth SWCMR (& collect samples for genetics=**Task2.9**)					X			X	X									
Task 2.7b Ground truth TAMR (& collect samples for genetics=**Task2.9**)						X				X	X							
Task 2.8a Collect GPS coordinates (same as **Tasks 2.7a-b**)					X	X		X	X	X	X	X						
Task 2.8b Develop larvae dispersal map (Claire Paris)													X	X	X	X	X	
Task 2.9 Assess genome-wide variance												X	X	X	X	X	X	X
Task 2.10 Report on nursery & outplant sites							X					X						X
Task 3.11a Install nurseries SWCMR								X	X									X
Task 3.11b Install nurseries TAMR						X				X	X							X
Task 3.12 Train MPA staff						X	X	X	X	X	X							X
Task 3.13 Monitor/maintain nurseries						X	X	X	X	X	X	X	X	X	X	X	X	X
Task 3.14 Report on established nurseries													X					X
Task 5.22 Regional/intl' dissemination		X			X						X							

Deliverables/Tasks/Activities	2018(YR3)											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Task 2.5a Community consultations SWCMR stakeholders									X			
Task 2.5b Community consultations TAMR stakeholders										X		
Task 2.6 Training event (3 days)	X											
Task 2.9 Assess genome-wide variance	X	X	X	X	X	X	X	X	X	X	X	X
Task 3.13 Monitor/maintain nurseries	X	X	X	X	X	X	X	X	X	X	X	X
Task 3.14 Report on established nurseries						X						X
Task 4.15 Outplanting				X	X							X
Task 4.16 Training (outplanting)	X											X
Task 4.17 Biodiversity monitoring	X	X	X	X	X	X	X	X	X	X	X	X
Task 4.18 Monitor outplant sites						X	X	X	X	X	X	X
Task 4.19 Report on outplanting						X						X
Task 5.22 Regional/intl' dissemination											X	

Deliverables/Tasks/Activities	2019(YR4)												2020
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
Task 2.6 Training event (3 days)	X												
Task 2.9 Assess genome-wide variance	X	X	X	X	X	X	X	X	X	X	X	X	
Task 3.13 Monitor/maintain nurseries	X	X	X	X	X	X	X	X	X	X	X	X	
Task 3.14 Report on established nurseries						X						X	
Task 4.15 Outplanting	X	X	X	X	X							X	
Task 4.16 Training (outplanting)	X	X	X	X	X							X	
Task 4.17 Biodiversity monitoring	X	X	X	X	X	X	X	X	X	X	X	X	
Task 4.18 Monitor outplant sites						X	X	X	X	X	X	X	
Task 4.19 Report on outplanting						X						X	
Task 5.21 National consults on results											X		X
Task 5.22 Regional/intl' dissemination											X		

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6.0 ANNEXES

6.1. LBCNP Site Evaluation

Coral Propagation and Reef Restoration in Placencia, Belize
Site Evaluation Report
Diego Lirman and Stephanie Schopmeyer
University of Miami
December 2014

Site Visit

Coral gardening sites in Placencia, Belize were visited by members of the Lirman Lab from the University of Miami in October 2014. During this visit, Dr. Diego Lirman and Ms. Stephanie Schopmeyer, experts in the field of coral reef restoration, conducted visual surveys of coral nurseries and coral restoration sites under the supervision of Ms. Lisa Carne, the Principle Investigator of the Belize coral restoration program. The purpose of this visit was to evaluate the status of coral propagation and coral restoration activities conducted to date, and provide recommendations for future steps including genetic analyses, site selection, monitoring methods, expansion of restoration activities, and the development of metrics of success.

The Placencia coral nurseries are well established and house **very healthy corals**. Based on the timelines provided by Ms. Carne and published data, the nurseries and reefs at Placencia appear to support some of the **fastest growth rates for staghorn corals in the Caribbean** (Figure 1). The coral nurseries visited presently hold a large number of staghorn and elkhorn fragments and colonies as well as a smaller number of massive coral species (Figure 1). Corals at the nurseries are grown in lines and frame structures similar to those used successfully in other nurseries in the region (Figure 1). Nursery frames are well maintained, which prevents loss of corals due to frame collapse or storms. **The corals being propagated exhibit low partial mortality, low predation impacts, and low disease prevalence**; all positive signs observed at both the nurseries and outplanting sites visited. The nurseries are located near natural reefs that provide easy access by reef herbivores that help control algal populations that may compete with nursery corals. In addition, the location of nursery sites in close proximity to outplant locations reduces stress on corals during transport from the nursery to restoration sites.

The highlight of our site assessment was our visit to **Laughing Bird Caye National Park** where Ms. Carne has been outplanting nursery-grown corals for years (Figure 2). **This site, in our opinion based on several site visits throughout the Caribbean and Florida, represents one of the best examples of successful large-scale reef restoration in the Caribbean.** The number, size, and health of transplanted corals of both staghorn and elkhorn make this site a huge success story. The large restored colonies provide a significant amount of topographical complexity and refuge habitat that are now serving as essential habitat for associated reef fish and macroinvertebrates (Figure 3).

This site represents clear evidence that propagation and restoration can be conducted at meaningful ecological scales and that **reef gardening is ready to be expanded into reef landscaping!** Nursery corals have survived the outplanting process and methods very well and have high survivorship up to at least 5 years after outplanting. Given the young age of the field of *Acropora* restoration, having examples of successful multi-year survival of nursery-grown corals is an important contribution of Ms. Carne's efforts. Currently, outplant sites show high

growth of both *A. cervicornis* and *A. palmata* with colonies exceeding the size required for potential spawning. The number and spacing of outplants provide good spatial coverage of the reef. The use of cement rosettes is very effective in securing the corals to the substrate as the corals overgrow the cement quickly. Additionally, outplanting corals of the same genotype to each rosette ensures that the corals will grow well together to form large colonies. The use of cement reduces the cost of outplanting since it is relatively cheap and readily available. Also, the use of cement reduces the need to use nails and plastic cable ties that are used by other programs. Lastly, wild populations of *Acropora cervicornis* and *A. palmata* in Placencia appear in good condition and we observed several large, dense thickets of staghorn coral that reminded us of the way these keystone reef-building species used to grow in shallow habitats of the Caribbean prior to their significant declines in the last few decades. Moreover, associated fish populations appear healthy, especially within the no-take areas visited.

Recommendations for Future Work

***It is important to note that the recommendations made in this section are solely based on the goals of: 1) scaling-up operations, 2) increasing efficiency, 3) filling knowledge gaps. These recommendations are made without knowledge of available financial resources and we fully recognize that all of these additional tasks will require considerable resources to be completed. Significant progress has already been made by Ms. Carne on several of our action items but we list them here nevertheless to highlight their importance in the expansion of restoration operations.*

Nursery Operations:

● The number of nursery platforms located at each nursery is appropriate for the level of resources/maintenance currently available. Without additional staff to maintain the nurseries, adding more platforms is not advised at this time. The amount of coral within each nursery was a bit high at the time of our visit to manage properly and avoid mortality due to frame collapse and fragment detachment. The program would benefit by having ~ 50% of the inventory outplanted onto wild reefs (current outplant sites or to new sites) as soon as resources allow. We were appraised by Ms. Carne that significant outplanting (up to 5000 fragments) had been completed after our visit!

● Corals within each nursery should be maintained at an appropriate nursery and/or outplanting size. Since acroporids benefit from pruning vigor, corals should be pruned regularly and tissue should either be used to increase nursery inventory or be directly used for outplating. In addition, larger colonies from the nursery may be used during outplanting activities. Corals that grow too large within the nursery are at risk of damage from storms, platform collapse, or disease/mortality. We suggest that a formal propagation and outplanting plan be drafted based on existing knowledge of the current and future inventory and growth and mortality rates, so that only corals that will be able to be fragmented and moved onto natural reefs are kept in nurseries. Having more tissue on nurseries that can be handled logistically is a potential problem that needs to be addressed through planning and additional staff (please see our section on staff/interns on this topic).

● Although the nurseries are well maintained, the number of corals currently located within each nursery is high while the number of distinct coral genotypes is low (compared to other programs that commonly house > 20 coral genotypes within a single nursery). We recommend additional

collections to increase the number of genotypes grown to serve as genetic repositories and provide additional parent genotypes for outplanting (keeping in mind that a diverse parent population is needed for successful gamete fertilization during spawning events). While the actual number of genotypes that can be kept in nurseries will depend on resources and the availability of parent genotypes from the local pool, we suggest no less than 10 genotypes of each species be kept within nurseries. Additional funds should be secured to genotype current inventory and new wild collections as needed (see following section on genotyping).

● The initial collections held at the Placencia nursery have been identified to genotype using microsatellites. More recently, an additional set of parent colonies (potential donor colonies for propagation) were sampled and sent to the US to be identified by Dr. Baums at Penn State. We recommend that all additional collections continue to be typed to be able to track the performance of individual coral genotypes. A new technique, genotyping by sequencing (GBS) has been applied to *Acropora* samples in Florida and elsewhere and can provide increased levels of genetic information. Microsatellite techniques for staghorn and elkhorn corals examine 6-7 loci. In comparison, GBS can analyze > 20,000 loci. The additional detail can be used to evaluate small differences in population structure and patterns of connectivity that are not possible with microsatellites. We recommend that Ms. Carne explore the possibility of analyzing all existing collections as well as future parent genotypes using GBS in partnership with US institutions like the University of Miami that are presently developing GBS techniques. While we recognize that these analyses will require additional expenses, the increased level of detail provided will fill important research gaps.

● Attention should be paid to the fate of host and symbiont genotypes. Future collections and outplanting activities should use genotypic information of both the hosts and symbionts to evaluate the role of coral and symbiont genotype on coral resilience. Ms. Carne has made significant progress on this topic and has already published one paper on the role of symbiont clade identity on coral growth. We suggest this work (requiring costly coral and zooxanthellae genetic analyses) be continued to identify holobiont winners/losers that can be used to further our understanding of Climate Change impacts on corals and coral reefs in the region

Ms. Carne has worked with Dr. Baker from the University of Miami on identifying the clade of zooxanthellae hosted by *Acropora* in Placencia. This research has shown that an unusual proportion of parent corals in Placencia harbor the thermo-tolerant D clade of zooxanthellae. Ms. Carne has shown that this association may indeed protect corals from thermal anomalies and may have already mitigated bleaching impacts. This finding has important implications for Climate Change adaptation so we encourage these activities to continue, recognizing that additional funds may be needed to continue to monitor both parent and symbiont genotypes.

● Additional nurseries may be established if there is a need to bridge spatial gaps either between existing nurseries or to have a source of corals closer to desired outplant locations. The location of wild *Acropora*, either isolated colonies or thickets, provide ideal focal points for bridging spatial gaps between existing coral populations. During our trip, we visited a potential new nursery location at South Silk Caye, located within the Gladden Spit and Silk Cayes Marine reserve (GSSCMR). We surveyed the reefs surrounding this emergent caye and, based on the location of the site, management status, the depth/habitats available, and the presence of remaining staghorn and elkhorn populations, **we fully support the decision to expand nursery and outplanting operations into S. Silk Caye in the near future**. When this new nursery is established, we suggest that coral genotypes now held at the other two nurseries be transferred to

S. Silk Caye. This new nursery should also hold the new parent genotypes being analyzed now by Penn State.

Out planting Operations:

● Outplanting should be expanded to include additional genotypes. While several genotypes have been outplanted to date by Ms. Carne within plots and within sites, we recommend that at least 5 additional genotypes of each species be added to existing sites as new collections grow (several new potential parent colonies were sampled during our visit and are now being genotyped in the US). We suggest that future sites be established with 5-10 genotypes of each species. The addition and tracking of new staghorn and elkhorn genotypes will represent an additional cost to the project, but increasing genotypic diversity is paramount for outplanting and future spawning success.

● Additional outplant sites should be chosen to bridge spatial gaps between existing populations. The addition of sites both within and outside protected areas can be used to ascertain the role of protection on restoration success. We fully support the use of S. Silk Caye as the next large-scale restoration site (see prior comment on new nursery locations). Its protected status and local stakeholder approval will likely make this new site as successful as Laughing Bird Caye National Park.

Monitoring:

● Because nursery and outplanting techniques have been very successful in Placencia, future outplantings should provide the type of data needed to quantify restoration success and establish regional benchmarks. Additional efforts should be made to quantify the amount of coral outplanted (total linear extension or size class and genotype) and growth over time. Simple measures of maximum width and height may be utilized to identify growth if total linear extension is too taxing. Corals may be binned into size classes (<10 cm, 11-25 cm, 26-50 cm, 50-100 cm, >100 cm, etc.) for easy identification and development. Additionally, measures of coral health, including partial mortality, disease, percent bleaching and recovery, and predation, should be monitored at least twice a year. Corals may be tagged to identify genotype for comparison of growth/survivorship. Suggestions for tagging include either colored cable ties at the base of the colony or placed into the cement or numeric aluminum tags nailed into the substrate near the colony (see section on metrics of success for further guidance). Several of these metrics have already been collected successfully by Ms. Carne, and we suggest these activities continue and expand as nursery and outpanting activities are scaled-up and new genotypes are added (please see Table 1 and later section for a full description of indicators of success).

● Documenting the successful sexual reproduction (spawning) of nursery-grown corals is an essential step in determining the long-term success of restoration activities. Ms. Carne has already completed one set of observations on staghorn spawning. We suggest that these efforts be continued on a yearly basis to document gamete formation and gamete release during the predicted spawning nights for both *Acropora* species. While just documenting the occurrence of spawning is an important outcome, additional data such as number of colonies/genotypes spawning and the size of spawning colonies would be an important contribution to the field.

Project Staff:

● Ms. Carne has been able to do a remarkable job with limited staff. She has been able to grow the Placencia project into one of the largest in the Caribbean region. The program has reached a

point where significant expansion is possible, but this will require the addition of permanent staff to support these activities. Work elsewhere in the Caribbean (especially work supported by IDB in the Dominican Republic in collaboration with the Punta Cana Ecological Foundation) has shown that coral restoration can provide alternative livelihoods for local stakeholders. Thus, the addition of permanent staff to Ms. Carne's project will serve the dual purpose of training local stakeholders and providing alternative livelihoods, and supporting the project expansion. **We encourage the funding agency to make the investment in new project hires at this critical time when propagation and restoration activities are ready to be expanded significantly!**
We also support the continued development of a student internship program supervised by Ms. Carne. Developing a formal internship program offering research opportunities to US or European students would increase the number of scientific projects that can be supported by the existing program and may provide additional funding to the project. Ms. Carne has done a remarkable job in establishing collaborations with coral reef experts in the US and the Placencia program can become an important research resource with additional funding needed to support expanded collaborations.

Regional Expansion:

● Considering the success of the nursery and out planting activities in Placencia and the resilience that these sites and coral genotypes have shown during recent temperature anomalies, it is recommended that the nursery collections be expanded with genotypes from sources outside Placencia. This would expand the role of the Placencia program as a genetic repository. In the future, when additional nursery programs are established by Ms. Carne at other locations in Belize, the Placencia genotypes can be also transferred to these new locations for safekeeping.

● The recovery of endangered coral populations needs to be based on regional, collaborative programs to reach ecologically meaningful scales. The success of the Placencia program should be leveraged to establish similar restoration centers across Belize so that recovering populations can reach the critical mass required to overcome spatial gaps in distribution of reproductive populations and the genetic alee effect of depleted parent stocks. Ms. Carne is uniquely positioned to lead such an effort through her successful program in Placencia and her contacts throughout Belize.

Metrics of Restoration and Propagation Success

Developing and quantifying metrics of success for coral propagation and restoration projects has been challenging and is a current focus of attention and research. One of our largest challenges has been to select a limited set of metrics that are: 1) easy to quantify, 2) have high precision and accuracy, 3) are ecologically meaningful, and 4) relate directly to ecosystem services that are the target of coral reef restoration.

While having one or two simple success indicators would be desirable, the complexity in the life history of corals often prevents this. Thus, we describe a set of hierarchical metrics to be measured at different stages of the propagation and restoration process and that can be evaluated in concert over the span of the project to assess success (Table 1).

Proposed Metrics of Success:

A) Nursery metrics
   1) Number of fragments in the nursery
   2) Number of distinct genotypes in the nursery
   3) Number of nurseries

B) Out-planting metrics
   4) Number of colonies out-planted
   5) Number of distinct genotypes out-planted
   6) Percent benthic cover of out-planted corals
   7) Spatial extent of out-planted populations
   8) Topographical complexity of out-planted populations
   9) Abundance, diversity, size of associated vertebrate and invertebrate organisms
   10) Number of sites restored

C) Individual-based metrics (to be measured at nursery and out planting sites)
   11) Fragment and colony survivorship
   12) Fragment and colony growth
   13) Disease and bleaching prevalence
   14) Predation impacts
   15) Sexual Reproduction (gamete development, gamete release, sexual recruitment)

Finally, it is important to note that the selection of evaluation metrics is only the first step in the assessment process. Indicator selection needs to be followed and supported by the establishment of benchmarks that can be used to track project status and trends, support site and genotype selection decisions, and compare metrics within and among project components.

Table 1 includes targets and suggested benchmarks for the different metrics. These are based on data collected outside of Belize and only presented here as initial suggestions. Benchmarks based on Belize-specific data need to be developed to track program success in the future. Many of these metrics have already been quantified by Ms. Carne in Placencia. Nevertheless, we provide these as a future guideline and to identify potential knowledge gaps that may be filled through project expansion.

Individual-based Metrics

Restoration and propagation programs around the Caribbean measure common colony-based metrics of success such as survivorship/mortality (as a binary metric or as a continuous metric in the case of partial tissue mortality) and growth. We highly recommend these two metrics (survivorship and growth) be recorded routinely as part of any collection, nursery deployment, and outplanting exercise so that values can be compared within the Placencia region as well as with other similar programs in Belize and elsewhere. We also recommend that both metrics be assessed within 1 month after collection/fragmentation/outplanting to assess the impacts of the methods used, and after 6-12 months to assess the adequacy of the site selected. Considering that both survivorship and growth are highly influenced by colony size, we suggest that these metrics be collected from fragments or colonies of similar or uniform size. We recognize that estimating these metrics can be time-consuming, especially when large cohorts are transferred. Therefore, we suggest that survivorship and mortality be assessed for a subset of fragments and colonies (no less than 15 per coral genotype). Colony growth is best assessed using the commonly accepted Total Linear Extension (TLE) method when colonies are small to medium (< 100 cm TLE). If larger colonies are used, then colony diameter can be estimated. If additional time is available, we suggest that number of branches be measured as an additional indicator of the growing environment.

Other colony-based indicators that are commonly collected by restoration programs include the prevalence of disease and bleaching, as well as the impacts of predation and damselfish occupation. These metrics can be assessed quickly on a subset of corals and are useful comparison metrics that can be used to compare the status and trends of both restored and natural populations.

The collection, archival, and analyses of these data will require the establishment of a database (Excel, Access, etc.). We suggest that resources be spent on designing a database that will increase the efficiency of these time-consuming tasks as the program grows. Regional programs like those run by The Nature Conservancy can serve as a model for such databases.

Community Metrics

While the majority of the metrics proposed are focused on the target species being propagated and restored, it is expected that the restoration of the target species will also recover ecosystem structure and function and will have positive impacts on reef-associated fauna and the services that healthy reefs provide. However, to be able to assess the potential beneficial impacts of coral restoration, adequate baselines are required to tease apart the effects of natural variability from the effects of restoration. The establishment of proper baselines will require a full documentation of community metrics prior to restoration as well as the establishment of un-restored site controls to be monitored along with restored sites through the recovery process. There isn't a rule of thumb that provides guidelines for the establishment and number of such sites and plots and preliminary assessments are needed to determine natural variability and to assess statistical power needed to determine desired levels of change/impacts that can be attributed directly to restoration.

The following two methods are being implemented by Mrs. Carne in Placencia. Thus our assessment is intended to provide some guidelines for data collection and implementation of these two methodologies.

Video Mosaics:

The PI has proposed the use of landscape mosaics for the documentation of thicket development and to assess the change in cover as colonies fragment and grow. Landscape mosaics are a powerful tool used to evaluate landscape-level changes in coral populations and will prove very effective for the documentation of population and community effects of restoration. Mosaics should be collected prior to outplanting as a baseline and should be repeated at 1 and 2 years to estimate changes in percent cover and thicket boundaries over time. The mosaics should be used to extract the following metrics right after outplanting:

1) Number (and status) of colonies within plot (visual counts)
2) Size of colonies within plot (colony diameters)
3) Distance between colony centers (distance measurements)
4) Percent Cover of restored species (point counts from random images)
5) Spatial extent of restored population (locate plot boundaries demarcated by out planted colonies)
6) Number and identity of non-restored benthic species in plot (visual counts)

By collecting these same mosaic-based metrics after 1 and 2 years, the practitioners should be able to track community status and trends efficiently. We suggest these mosaic-based metrics are supplemented by diver-based colony height measurements and visual surveys of fish and invertebrates (see next section).

Fish and Invertebrate Surveys:

A number of reef fish survey methods are being used by programs around the world. Due to the relatively limited spatial footprint of restoration activities, we suggest that plot-based methods are used instead of large-scale roving diver methods. Two methods commonly used for reef fish are the AGRRA and RVC methods. Any of these two methods will provide status and trends data for reef fish populations. As mentioned already, it is important that adequate controls are included in the survey protocols to be able to ascertain the effects of restoration activities. Plots centered on the restored areas should be paired with surveys of unrestored plots in the same habitats. Due to the complex morphology of *Acropora* colonies used for restoration, it is also recommended that more detailed surveys that focus on cryptic and small fish and invertebrate species are used to expand on the mobile species documented during the AGRRA or RVC surveys. A spacing of at least 100 m is suggested to ensure independence of survey plots (at least for species with limited ranges). Due to the high variability in fish recruitment patterns, we suggest that fish surveys be conducted at least twice per year.