Research directions for 2020 to Achieve Scaled Reef Restoration in alignment with our long-term goal 3

For Iberostar, our goal for reef restoration is (in order of importance and prioritization) to enhance coastal protection, increase fish biomass (if possible, fish biomass important for local food security), and optimize local biodiversity. We aim to do so while optimizing resilience to expected threats from climate change at scale in at least the locations where our operations persist reaching an upper limit of the 5.6 square kilometers of reef that surround our hotels. In 2020, we have 6 specific research projects that allow us to take steps towards achieving this goal.

1. **Assessing and genotyping threatened staghorn coral *Acropora cervicornis* nurseries during restoration in southeast Dominican Republic**: Findings from this study have helped us to establish the baseline genetic diversity of the program in the Dominican Republic (though tracking of strains has been lost due to hurricane damage), as well as overall growth rates, survival, and common causes of mortality of coral restoration work in the southeast of the Dominican Republic.
2. **Functional importance of *Acropora cervicornis* in outplanting sites**: Findings from this study have allowed us to demonstrate that fish biomass can increase with consistent outplanting of *Acropora cervicornis*, thus helping to achieve our secondary goal of optimizing fish biomass with a focus on species for food security.
3. **Scaling reef restoration with Iberostar's Wave of Change: leveraging existing capacity to explore commonalities in situ and land based nurseries**: Findings from this study will allow us to present our efforts on scaling and standardizing reef-restoration practices in at least four locations as well as present the consistent abiotic and biotic indicators we expect to measure at each site. It will present the species and methods that we use or intend to use, partnerships that have, and logistical framework we use for scaling our efforts within Iberostar.
4. **Assessing the role of outplanting density on growth and survival of transplanted *Acropora cervicornis* in Coco Reef, Bayahibe**: Findings from this study will allow us to verify findings on optimal planting density as well as compare methodologies for outplanting used by partners in Mexico and the Dominican Republic to evaluate success of methodology in one location. This allows us to develop standards across our network of restoration initiatives.
5. **Defining bleaching thresholds of Caribbean corals and selecting individuals for climate change resilient reef restoration**: Findings from this study will allow us to characterize phenotypic diversity within parent colonies grown in nurseries. This allows us to better curate resilient stock of sufficient individuals to represent regional genetic diversity as we work towards restoration for coastal protection, fish biomass, and overall biodiversity.
6. **Testing a field-based method for approximate genotyping of *Acropora***: From this study, we test the viability of a field-based, inexpensive approximate genotyping method.

If the method is successful, it will assist in recovery of stock organization after a hurricane and will allow us to be efficient in our genetic sampling of parent colonies. We also use this study to establish and maintain the genetic reservoir of Acropora cervicornis in the Dominican Republic.

Assessing and genotyping threatened staghorn coral *Acropora cervicornis* nurseries during restoration in southeast Dominican Republic

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Introduction

Acropora cervicornis is a key Caribbean species, both in structural and functional terms. It has suffered an important population loss since the 1980s and exhibited no signs of recovery in the following three generations (Aronson and Precht, 2001; Chamberland et al., 2013). Hence, it is classified as a Critically Endangered Species (Aronson et al., 2008). Its fast growth rate compared to other species makes it a perfect candidate for use in active coral restoration programs (Young et al., 2012). In 2011 Fundación Dominicana de Estudios Marinos (Dominican Marine Studies Foundation, FUNDEMAR) started the *A. cervicornis* restoration program at Bayahibe in the southeastern part of the island (Calle-Triviño et al., 2020 *in rev.*). To assess the performance of the *A. cervicornis* restoration program growth and survival from 2011 to 2017 were evaluated. Analysis and interpretation of results were based on the relative yielding (mean) of each nursery and outplanting site, using the stoplight model proposed by Schopmeyer et al. (2017). We documented the results of the program during non-stress conditions and under stress scenarios caused by the strong cyclonic seasons of 2016 and 2017 in Bayahibe in southwestern Dominican Republic. In addition, we include genotypic characterization of the "mother nursery", considering the evolutionary objective of restoration efforts to establish populations of self-sufficient corals and sexual reproduction that have sufficient genetic and phenotypic variation to adapt to changing environments (Baums et al., 2019), it is vitally important to have genotyped nurseries in which different genotypes and phenotypes are identified and to take advantage of genes and specific traits in future restoration.

Hypotheses

1. In-water coral nurseries and outplanting sites assessed in the restoration programme will have high survival and annual productivity values.
2. Genotype characterization results suggest that there is a "genetic stock" with enough genetic diversity to continue developing the program.
3. Having several in-water nurseries in the same area of influence will increase the chances of survival from disturbances such as hurricanes and storms.

Expected results

Here we show that mean survival of the fragments for 12 months was 87.45 ± 4.85% and mean productivity was 4.01 ± 1.88 cm year^{-1} for the eight nurseries. Mean survival of outplanted colonies during 12 months for six outplanting sites was 71.55 ± 10.4% and mean productivity was 3.03 ± 1.30 cm year^{-1}. The most common cause of mortality during the first 12 months, both from the nurseries and the outplanting sites, was predation by *Hermodice carunculata* fireworm. We identified 32 multilocus genotypes from 145 analyzed individuals. The results and techniques described here will help to continue developing current and future restoration programs in nurseries and outplanting sites.

Functional importance of *Acropora cervicornis* in outplanting sites

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(In preparation for submission in Frontiers)

Introduction

In the Caribbean, work has been done on coral restoration with threatened staghorn coral (Lirman and Schopmeyer, 2016). However, most of the studies have been focused on evaluating the growth and survival of colonies in nurseries and in outplanting sites, to describe the "success" of the programmes of restoration (Schopmeyer et al., 2017). The purpose of this study was to identify the ecological processes of succession and how ecosystem functions are altered in outplanting sites in Bayahibe. For this purpose, three outplanting sites and a control zone were monitored for one year in order to determine the percentage of the benthic cover and fish biomass. Subsequently we developed multivariate analysis in conjunction with the coefficient of functionality of the coral species present in the three outplanting sites, to determine the increase in some variables, considered as a positive effect due to outplanting actions.

Hypothesis

1. The consistent outplanting of a single coral species over the period of one year time, in this case of A. cervicornis can increase the functionality of the structural three-dimensionality and thus the biomass of fish and the coefficient of functionality in the outplanting sites.

Expected results

In general, for the three outplanting sites and the control zone we found 28 species of coral. Concerning the fish community, the family Scaridae showed the highest abundances for all three outplanting sites and the control zone, followed by the family Acanthuridae.

As efforts in the outplanting sites intensified, Reef Functional Index (RFI) increased directly proportional to the increase in percentage of coral cover. At the same time, the percentage of macroalgae cover and abiotic substrate available for colonization decreased.

We found a direct correlation by increasing the number of transplanted colonies in each of the areas with the increase in fish biomass and the increase in the coefficient of functionality for the three outplanting transplant areas. Demonstrating that the role of *A. cervicornis* has very important direct implications for restoration efforts.

The increased coverage of *A. cervicornis* is directly proportional to the increase in the three-dimensionality and structural complexity of the ecosystem which in turn offers a greater number of refugees and feed for other commercially and/or ecologically important invertebrates (eg: octopus, lobsters) and of course for reef fish. This is why the observation of the dynamics of fish communities after transplants is fundamental to understanding the ecology of the system and evaluating the "quick" contributions of the restored sites.

Scaling reef restoration with Iberostar's Wave of Change: leveraging existing capacity to explore commonalities in *in situ* and land based nurseries

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Introduction

In recent decades there has been a reported decline in the abundance of corals worldwide (Burke and Maidens, 2005; D'agata et al., 2014; Anthony et al., 2015; Pendleton et al., 2016; Hughes et al., 2017; Hughes et al., 2018; Lamb et al., 2018). As part of a strategy to reduce the degradation of reefs and contribute to the recovery of diminished coral populations, coral reef restoration programs have been established in different parts of the world (Rinkevich, 2005; Precht, 2006; Petersen et al., 2007; Edwards and Gómez, 2007; Edwards, 2010; Johnson et al., 2011; Nakamura et al., 2011; Young et al., 2012; Toh et al., 2012; Chamberland et al., 2015, Rinkevich, 2015; Lirman and Schopmeyer, 2016; Schopmeyer et al., 2017; Calle-Triviño et al., 2018). However, major gaps still remain for performing restoration for ecosystem function at scale, a gap that is increasingly urgent to resolve in the declining reefs of the Caribbean. The private sector has been viewed as a potential candidate for providing investment and new solutions.

Hypothesis

1. Coral development will depend on the physical and chemical conditions and parameters at each location.

Expected Results

Here, we will present results of a multi-species network of restoration programs across the Caribbean with "Wave of Change" Iberostar's movement to contribute to active programs in resilient reef restoration towards coastal protection where we operate by 2025. In the Dominican

Republic currently we have an in situ coral nursery organized by genotype, with 38 structures capable of supporting an average of 30 fragments of coral each. We have a Coral Lab in the Bavaro Hotel land facilities with a genetic stock of 12 coral species and eight raceways individual control systems to simulate bleaching events where we have already begun to make our first thermal tolerance tests across different genotypes. In Aruba we're collaborating for the maintenance of a coral nursery in situ and are in the process of developing a second Coral Lab to house coral on land for additional research and outreach. In Mexico we are installing our first in situ coral nursery in Cozumel and Playa Paraíso. We will have genetic stock available in three nurseries for the development of different activities both education, research, technological innovation, recreation and tourism. At the regional level, the project will improve understanding of how to use coral reef restoration as a tool for adaptation to climate change, we will also present techniques that work in all three geographies, taking into account political environments, species biology and their local adaptations, so that we can provide models for sustainable management of reef ecosystems, and build capacity for restoration and long-term management, particularly in partnership with the private sector. The presence and abundance of corals will be determined by the interactions between the physical-chemical and biological factors of the habitat at both the macro and micro scales and the intrinsic characteristics (life history) of each of the species.

Assessing the role of outplanting density on growth and survival of transplanted *Acropora cervicornis* in Bayahibe

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Introduction

In the face of the worldwide accelerated deterioration of coral reefs, different management and conservation strategies have been implemented, including the development of coral restoration programs (de la Cruz & Harrison, 2017). Although significant efforts have been made in the south-east of the Dominican Republic since 2011, there are not yet standardized methodologies for outplanting coral with high growth and survival rates after one year.

As part of the Wave of Change movement to provide science-based solutions, we test two techniques used in different restoration programs as well as test different planting densities. Further, we will measure different physical-chemical and biological parameters such as Nutrients, Ph, Salinity, Temperature, Structural complexity, Coral cover, Coral size structure, Outplant health, Outplant survivorship and Outplant growth rate. We aim for this study to indicate the most suitable planting density and methodology in this particular site.

Hypothesis

1. We expect there to be differences in survival in outplanting density
2. We expect for outplanting methodologies to have differing survival rates for outplanted coral

Expected Results

According to the Reef Resilience Network much research has been conducted to determine which transplant designs (such as density, spacing and disposition) maximize survival and growth of branching coral transplants. However, there does not yet appear to be a "best" design.

We expect planting density at around 3-6 individuals per square meter will produce the highest survival rates (Ladd et al., 2016, Frías-Torres et al., 2018).
We expect the outplanting technique using cement bases will have higher survival rates than outplanting with nails.

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Defining bleaching thresholds of Caribbean corals and selecting individuals for climate change resilient reef restoration

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Introduction

Bleaching thresholds have been identified at different spatial scales to predict the future of coral reefs facing climate change (van Hooidonk and Huber, 2015; Smith et al., 2015). However, few models have included variation in susceptibility among coral taxa (Swain, 2016; 2017). This information is fundamental to predicting future coral community structure, as taxonomic differences may play an important role in redesigning the species composition. Despite the number of factors involved in the bleaching response (Mydlarz et al., 2010), consistent differences in this response have been reported between coral species (Grottoli et al., 2006; Guest et al., 2016; Singh et al., 2019), with species that can resist greater heat-stress being more likely to persist under future ocean warming (Hughes et al., 2017; Loya et al., 2001; Marshall et al., 2000). Moreover, studies have shown that heat tolerance can also vary among individuals of the same species (Lohr et al., 2017; Morikawa and Palumbi, 2019; Shaw et al., 2016). Maximum stress temperature withstood by species can be defined as the temperature at which 50% of the bleaching response is observed (T50) (Morikawa and Palumbi, 2019). There is a need to establish the bleaching temperature of species in the Caribbean, as most studies have focused on those in the Pacific (Levas et al., 2018; Marshall et al., 2000).

The aim of this study is to determine the T50 temperature of six common reef-building species in the Caribbean: *Acropora cervicornis*, *Diploria labyrinthiformis*, *Montastraea cavernosa, Orbicella annularis, Orbicella faveolata and Porites porites*. Additionally, intraspecific thermal tolerance will be assessed in order to find thermally resilient individuals within each species at their T50 temperature. This will assist in our capacity to characterize the phenotypes of coral for climate-resilient reef restoration. Samples for interspecific variability will be collected for 60 unique colonies across the 6 species in the Southern region of the Dominican Republic. Intraspecific variability in thermal tolerance will be then assessed using another set of 20 different colonies per species .Thermal tolerance will be assessed conducting 3 hour heat pulse experiments with the temperature control system at Iberostar's Coral Lab . The control system consists of four 120L-experimental tanks individually connected to four sump tanks containing a warm-and-cold-water interchanger controlled by a programmable logic controller (PLC) with a 0.01°C accuracy. Flow through rate of new water will be set at 60 L/h and water will recirculate between sump and main tank at a rate of 600 L/h. Control tanks will be set at 30°C and heat pulse experimental temperatures will range between 33 to 36°C depending on the species of study. 12/12h light/dark cycle will be provided using LED illumination. Bleaching response will be visually ranked as well as measured spectrophotometrically using chlorophyll content as a proxy.

Hypotheses

1. The temperature at which fifty percent of the colonies will bleach (temperature T50) will vary across species.
2. Within each species, there will be sufficient variability in the response to the predetermined T50, with some fragments bleaching sooner (sensitive genotypes) than others, that may bleach later, less or not bleach at all (resistant genotypes).

Expected results

Establishing the temperature at which half of the colonies of a species bleach (T50) will allow us to compare the heat tolerance of different caribbean species in a controlled heat pulse (interspecific variability).

This will reveal some of the species whose chances of composing future coral communities are higher (winners), and some of those whose permanence could be at risk (loosers) (Loya et al., 2001). Changes in coral community composition will also imply functional changes in the ecosystem. For instance, a reef with *Porites astreoides* as the most abundant species, whose structural complexity is low, will likely sustain less biodiversity than a reef containing other highly-complex branching species such as the acroporids (Gonzalez-Barrios and Alvarez-Philipp, 2018).

Interspecific variability will thus highlight the need for reef restoration to diversify to include multiple species. Despite the bleaching threshold of a reef should increase once the most vulnerable species have disappeared, decreased biological diversity will promote the decline of the overall resilience to disturbance (Van Hooidonk and Huber, 2009).

Further, we hope to unveil the intraspecific variability within different colonies of a species at their designated T50. Morikawa & Palumbi demonstrated this information could be used to curate a coral nursery (of multiple species) that was more likely to survive multiple bleaching events. This experiment has not been replicated for species in the Caribbean. If successful, and if sufficient baseline genetic diversity is present (Calle Trivino in review), then we aim for these findings to inform our restoration efforts. .

Expected intraspecific variability will emphasize the implications that selecting locally discovered thermally resilient individuals could have in reef restoration practices, since more tolerant individuals can improve survival rates thus increasing overall success of reef restoration in a changing climate (Bowden-Kerby and Carne, 2012; Drury et al., 2017, Morikawa and Palumbi, 2019). By identifying intraspecific diversity amongst species, we boost overall biodiversity and ensure that species such as Acroporids, which have been historically vulnerable to bleaching, can be maintained in restoration efforts. Here we show it was possible to assay standing diversity with simple tools to find the winners and losers of bleaching before it occurred for 5 species. Through this study we hope to be instrumental in the prediction of coral community structure under future warming conditions and to contribute towards more effective restoration practices.

Testing a field-based method for approximate genotyping of *Acropora.*

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Introduction

The threat posed to the world's coral reefs by anthropogenic stressors such as climate change, overfishing and pollution is on the rise (Hoegh-Gulberg et al., 2007; Hughes et al., 2003; 2017) Reefs are predicted to keep on declining, as degradation rates cannot be met by rates of natural recovery processes (Donner et al., 2005; Young et al., 2012). Coral reef restoration has recently gained popularity as a practice with high potential to help mitigation of declining patterns and recovery of damaged or depleted reefs (Wilkinson, 2008; Young et al., 2012). Despite successful biomass increases in nurseries, efforts are still vulnerable to the accentuating threats of climate change. There is a need of improving restoration practices in order to promote more resilient reefs (Van Oppen et al., 2014; Wilkinson, 2008). To do so, efforts should focus on enhancing genetic diversity (Shearer et al., 2009; Van Oppen and Gates, 2006). The greater it is the variation of alleles in the population, the more likely would be for some individuals carrying certain alleles to survive the new conditions of their environment, and hence, the more chances will have their population to adapt (Bay, 2017; Drury et al., 2017; Reed & Frankham, 2003) Moreover, selecting genotypes that show higher thermo tolerance in lab experiments have been proved to increase survival rates in coral nurseries (Morikawa and Palumbi, 2019)

It is only recently when genetic and genotypic diversity have been considered in reef restoration (Baums, 2008; Shearer, 2009; Young, 2012). However, this is still a challenge, as in most cases, existing genotypic diversity in nurseries or wild populations is unknown and the current tools to assess it are expensive. Genetic markers such as microsatellites and Single Nucleotide Polymorphism (SNP) have been used to survey coral diversity (Lundgren, 2013). A potential easy way of assessing the existing genotypic diversity in a nursery or population arises from the basis that the living tissue and skeleton of two ramets from the same genet (i.e. two fragments of the same genotype) will fused when grown in contact with each other (Hughes & Jackson, 1980; Neigel, 1983). This process is known as isogenic fusion and can be an important strategy for small coral fragments to have more access to shared resources and to be less vulnerable in terms of spatial competition and environmental disturbances (Forsman et al., 2015).

The aim of this study is to demonstrate the viability of the "fusion method" as a potential tool to assess genotypic diversity in coral populations. A number of tests will be carried out at Iberostar´s Coral Lab. The tests will consist of fragments of *Acropora cervicornis* from different ramets that will be set to grow together. Following fusion between fragments will be examined under a dissecting microscope. Results will be compared to those of DNA analyses conducted on 60 of our nursery ramets by Morikawa et al. in order to assess viability of the method.

Hypothesis

1. When growing close together, clonal fragments will fuse their tissue, whereas fragments from different parent colonies will create a fissure in between. This simple fusion-fission

method can be a strong tool in estimating genotypic diversity, with comparable accuracy to that of results from laboratory genotyping analyses.

Expected results

Clonal fragments are expected to undergo a self-recognition phenomena that will precede the fusion of their tissue (Neigel, 1983). Self-recognition phenomena of the genus *Acropora* was first described by Hildemann et al. (1975). In contrast with this “acceptance response”, contact between non-clonal fragments is expected to result in the formation of a “rejection response”, described in Neigel (1983) as a suture line at the skeletal interface separating the tissues of the two fragments. Bleaching, anomalous growth and incomplete development of the polyps are also commonly found around this suture.

Northdurft & Webb (2012) interpreted fused fragments of *Acropora sp*. to belong to the same genotype despite stating that no genetic analyses had tested that hypothesis, since studies suggest that it is unlikely that two mature non-clonal fragments would have fused at both tissue and skeletal level. Forsman et al. (2015) proved fusion of small clonal fragments of *Orbicella faveolata, Pseudodiploria clivosa* and *Porites lobata* in aquarium conditions.

It is expected that both the clones and unique genotypes determined through fusion and fission method will correspond to those determined by DNA analysis.

Fusion and fission method could be a useful method to assess genetic diversity of *Acropora cervicornis i*n coral nurseries without the need of carrying out expensive DNA analyses in the lab. This would be extremely advantageous for reef restoration operations, since not only the number of genotypes composing their nursery could be estimated, but also it will allow practitioners to grow genotypes separately and keep track of their different traits (such as growth rate, survival rate, disease resistance…). This will help choosing genotypes for transplanting and making sure genetic diversity is maintained when transplanting.

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