**PROJECT SUMMARY**

**Overview** Coral reefs are declining worldwide as a result of local and global factors including the effects of climate change. This decline is marked in Caribbean reefs where coral cover is now below 15% in many areas. Conservation efforts are urgently needed to reduce such loss, recover depleted populations and restore natural habitats. A popular measure to boost coral populations is coral restoration. Coral restoration involves the recruiting of fragments that generate coral stocks at farms, and the subsequent transplant to various reef habitats. During restoration, coral fragments often survive depending on the habitat they are transplanted to and survivorship is enhanced when fragments are used to repopulate nearby areas than when transported to other locations. Transplantation to different depths within a reef increases mortality by at least 30%. Even colonies moved to the same habitat but to different locations suffer large (> 40%) mortalities. When colonies do not die, corals grow less and perform sub-optimally in the new location Our goal with the proposed project is to systematically estimate the role of natural selection acting on coral populations of the star mountainous coral (*Orbicella faveolata*) at different depths by estimating survivorship rates in the wild and by analyzing patterns of genomic variation.

**Intellectual Merit** The proposed study involves coral reef restoration efforts by bridging experimental and evolutionary biology in order to better understand coral adaptation to depth and its direct applicability in the field of conservation genomics. By searching for genomic signatures of adaptation to depth and survival in the wild through reciprocal transplant experiments we aim to further improve our understanding of the mechanisms that promote and maintain biodiversity through adaptive processes on coral reefs. Whether deep reefs can act as a source of colonies for future restoration initiatives, despite strong segregation along depth clines, could provide an alternative to repopulate dwindling coral populations across the Caribbean basin. The reef building coral *O. faveolata* is an ideal species to address adaptive divergence and coral restoration because is a dominant and widely distributed species across the Caribbean and has been the focus of multiple studies concerning its biology and ecology.

**Broader impacts** Many corals, including the *Orbicella* species complex, have been listed as endangered under the Endangered Species Act (ESA). With the severe decline of *O. faveolata* populations, recovery of this major Caribbean coral reef builder is paramount. Successful restoration depends on whether corals are able to thrive in transplanted sites by altering phenotypic traits, which often are underlined by genetic variation. The core of our proposal addresses a conservation issue with the goal of accelerating the recovery of these important Caribbean reef builders. A major general recent criticism from the public to basic science is whether such knowledge would translate into tangible benefits for society. In this project, we will build upon decades of study on coral adaptation to enhance restoration efforts of coral populations.Coral reefs need to be rapidly restored worldwide. Our study on the genomics of adaptation to depth in corals would bring scientists and professionals in coral farming together and implement cutting edge basic science findings into already established coral farms in Puerto Rico (NOAA) and the US Virgin Islands (The Nature Conservancy, TNC). The TNC facility is consulting with us to scale up to multiple species beyond their current efforts with branching corals. Our study will be a pilot, unparalleled detailed population genomics effort for *O. faveolata* that will help shape the TNC’s strategy to expand to other brain and mound Caribbean corals.

**1. Background**

Depth is widely recognized as a major segregating factor in marine benthic communities, particularly in sessile taxa such as coral, as it provides multiple axes of niche diversification (Knowlton 1993, Knowlton and Jackson 1994, Kirk et al. 2009, Serrano 2013). As a result of such stratification, and prior to widespread coral reef degradation, reefs were often classified by zones according to species presence at particular habitats (Goreau 1959). Such stratification is often mirrored by segregation of sister species (Carlon and Budd 2002, Lesser et al. 2010, van Oppen et al. 2011, Bongaerts et al. 2013). Corals at different depths experience differences in exposure to solar irradiance, waves and currents force, nutrient and sediment regimes, distribution of predators and mutualists, and availability and composition of food sources (Knowlton and Jackson 1994). In response to this multidimensional environmental variation, species or populations differ in their morphologies (Budd and Pandolfi 2004, Fukami et al. 2004, Prada et al. 2008, Budd and Pandolfi 2010), feeding strategies (Lasker et al. 1983, Lesser et al. 2010), physiology (Lesser et al. 2010), symbiotic relationships (Kirk et al. 2009, Prada et al. 2014), and resistance to predators (West et al. 1993). Understanding adaptation to depth is urgently needed as deep-water reefs (> 30m) could act as larvae reservoirs to repopulate neighboring and extensively deteriorated shallower reefs (Bongaerts et al. 2010). Testing whether shallow and deep reefs experience gene flow, and if deep residents survive and grow in shallow habitats, would add extra support to this ‘deep reef refugia’ hypothesis and could provide a ray of hope for coral restoration.

A plausible explanation for corals` specialization along a depth cline is that populations in different environments experience local adaptation, thus immigrants to a different habitat suffer reduced fitness and increased mortality, a process known as immigrant inviability (Nosil et al. 2005). Such inviability in corals is stronger as there is limited recruitment success of larvae (< 2% a year) and high survivorship of adults (94% per year) (Yoshioka and Yoshioka 1991, Yoshioka 1998, 2009, Prada and Hellberg 2013). To reach adulthood, a settler must successfully sort multiple ecological challenges until it achieves a minimum reproductive size (at least 5 years). This large window between recruitment and sexual reproduction creates a strong ecological filter and therefore a selective force (Prada and Hellberg 2013). Moreover, this long period greatly reduces genetic connectivity between depth-adapted populations and enhances the opportunity for habitat-specific selection and divergence. On coral reefs there are many examples of divergence between depth-segregated populations. Some of the examples of divergence between depth-segregated populations include the brooding corals *Favia fragum* from Panama (Carlon and Budd 2002), *Agaricia fragilis* (Bongaerts et al. 2017) and *Porites astreoides* in Florida and US Virgin Islands (Serrano et al. 2016), *Seriatopora hystrix* and *Pocillopora damicornis* from the Great Barrier Reef (Bongaerts et al. 2010b, van Oppen et al. 2018) and the broadcast spawner *Montastraea cavernosa* (Serrano et al. 2014). Genetic divergence across depths has also been reported in the Mediterranean anthozoans *Corallium rubrum* (Costantini et al. 2011) and *Eunicella singularis* (Costantini et al. 2016).

More recently, preliminary evidence from the mountainous star coral (*Orbicella faveolata*), a major Caribbean reef builder species listed as threatened under the Endangered Species Act (ESA), also suggests genetic differentiation across depths in Puerto Rico (Fig.1). Several aspects of this species make it ideal to address adaptive divergence and coral restoration: 1) it is widely distributed across the Caribbean basin (Lasker and Coffroth 1983, Weil and Knowlton 1994, Fukami et al. 2004, Pandolfi and Budd 2008, Prada et al. 2008b), 2) along depth gradients from 1 m up to at least 50 m, (Knowlton et al. 1992, Weil and Knowlton 1994, Pandolfi and Budd 2008, Prada et al. 2008b) 3) there are multiple studies detailing its ecology and physiology both in the field and aquaria (Colombo- Pallotta et al. 2010, DeSalvo et al. 2010, Voolstra et al. 2011), 4) reciprocally transplanted colonies show differential survivorship (Prada et al. 2008b, Prada and Hellberg 2013) and 5) there is an assembled and annotated reference genome (Prada et al. 2016).

**Figure 1.** 117 of over ~91k markers from our pilot study of *O. faveolata* in depth-segregated populations in Puerto Rico are likely under selection as suggested by BayeScan (Left;FDR 0.05). The outlier loci show large allele frequency differences between populations of adults (right). We are showing twenty aexemplar of the *FST*  outliers.

**2. Specific Aims** For deep habitats to act as source of larvae to shallow ones, genetic connectivity across habitats needs to be high and survivorship rates of deep larvae on shallow reefs has to be equal or higher than that of their shallow counterparts. Unfortunately, colonies survive less when transplanted to opposite depths. This, together with genetic differentiation, that suggests limited connectivity, has been documented in coral populations living at different depths (Bongaerts et al. 2010b, Prada and Hellberg 2013). We hypothesize that adaptation in coral populations is largely due to differences in fitness of alternative alleles in the wild, an idea coming from previous studies (Prada and Hellberg 2014). Our goal with the proposed project is to systematically estimate the role of natural selection acting on coral populations of *O. faveolata* at different depths by estimating survivorship rates in the wild and analyzing patterns of genomic variation in the coral host and its dinoflagellate symbiont.

**Aim 1.** Quantify the strength of selection at the genetic level in natural populations using a reciprocal transplant experiment and establish the identity and stability of dinoflagellate symbiont associations.

**Aim 2.** Infer the strength of selection from distribution of allelic frequencies across depths.

**3. Estimating the strength of selection through a reciprocal transplant experiment** **(Aim 1)** We will first search for signatures of natural selection via genome scans across four depth-segregated replicated populations –Puerto Rico (PR), US Virgin Islands (USVI), Curaçao (CU) and Panama (PA).We hypothesize that shallow and deep populations are the byproducts of genetic variation that has been filtered by natural selection. We thus expect that loci under selection present elevated genetic differentiation (higher *FST*) than the neutrally evolving genomic background. We expect the signature of selection to be spread within a few kilobases of the selective region, which will allow us to more accurately identify genomic signatures by using sliding window approaches (Reid et al. 2016). We also assume that alleles under selection would have different fitness values in shallow and deep areas. A colony that carries an allele that provides an advantage for being in shallow areas will have a higher probability of survivorship in shallow areas than a colony that carries an alternative allele. Secondly, we will look for Symbyodinacea markers that map to the *Breviolum minutum* genome (LaJeunesse et al. 2018), previously known as *Symbiodinium minutum* (Shoguchi et al. 2013), and other genomes and transcriptomes for other Symbyodinacea (Liu et al. 2018) to identify the predominant symbiotic association in each population and depth. This could allow us to link symbiont variation to adaptation to depth in this species. Thirdly, we will calculate allele frequencies before and after transplantation and estimate the strength of selection at two locations (PR and USVI). To generate the transplants, we will collect 50 fragments from shallow areas (< 5 m) divide them and place one back in a shallow environment, place the other one in a deeper habitat (~25 m) and preserve a sample for DNA. Lastly, we will test for changes in Symbyodinacea on the coral host after transplantation to determine the stability of the symbiotic relationship.

To identify regions of the genome under selection, we will take two approaches. First, we will localize targets of selection using the software OUT-FLANK (Whitlock and Lotterhos 2015), which calculates a likelihood based on a trimmed distribution of *FST* values to infer the distribution of a *FST* for neutral markers. Second, we will detect outlier SNPs with BAYESCAN v. 2.1 (Foll and Gaggiotti 2008), a Bayesian method based on a logistic regression model that separates locus-specific effects of selection (‘adaptive’ genetic variation) from population-specific effects of demography (‘neutral’ genetic variation). Third, loci will be screened with *pcadapt*, an R package to perform genome scans for selection based on principal component analysis (Luu et al., 2017). We will also identify signatures of selection by a *FST* sliding window approach using Weir and Cockerham’s method (Reynolds et al. 1983) as implemented in VCFLIB (https://github.com/vcflib/vcflib). If our data allows it (our aims do not depend on it), we will use information in extended haplotypes to test for selection via the haplotype statistic based on soft and hard sweeps (nsL) (Ferrer-Admetlla et al. 2014). We will use this over other metrics as it does not require phased data. The initial SNP candidates would be furthered validated by looking at: 1) loci scored as outliers by at least two methods and whether or not different populations at the same depth have the same highly differentiated SNPs, 2) and changes in allele frequencies in transplanted colonies as a result of mortality through a permutation approach.

**4. Strength selection inference from allelic clines across depths (Aim 2)** Alleles associated with adaptation to depth should change in frequency according to the depth gradient. The slope of that change is correlated with the strength of selection. We will estimate allele frequencies by genotyping individuals across six depth strata across two locations (PR and USVI). Loci with steep clines –putatively adaptive- should coincide with candidates loci identified in aim 1.Selection against unfit migrants such as in immigrant inviability should generate allelic clines because selection is acting differentially across environmental gradients (Endler 1975, Slatkin 1975, Endler 1977). The graphical representation of a cline is one in which the frequency of an allele under selection changes gradually as one moves from one extreme of the gradient to the other. If selection is strong, we expect stepped clines (Barton 1983). Conversely for neutral loci, no-deviations in allele frequency (on average) should be detected across the gradient, generating a flat line with little to no slope (Fig. 2). We also expect that linkage disequilibrium (the association among loci) at the center of the cline to be highest and decline towards the edges (Barton and Gale 1993). To determine the shape of a cline, one can use mathematical sigmoidal descriptions and estimate the center of a cline and its width (Endler 1977, Szymura and Barton 1986, 1991). Given that selection is strong to remove maladapted colonies in depth segregated-corals (s > 0.3) (Prada and Hellberg 2013, Prada and Hellberg 2014), we expect very steep clines for adaptive alleles.

To construct clines for each locus we will sample individuals across a transect from shallow to deep in both PR and USVI. We will collect 20 samples per species at each of six depths (< 5m, >5m and <10m, > 10m and < 14m, > 14m and < 19 m, > 19 m and < 25 m and > 25 m). Since shallowest and deepest samples come from our transplant experiment, we will have 160 samples (40 samples from < 5m, 40 samples from > 25m, and 20 each for the four depths in between). To identify loci with sharp clines we will run three analysis: 1) We will use the Bayesian genomic cline approach as implemented in *bgc* (Gompert and Buerkle 2011), 2) we will use a binomial logistic regression of allele frequencies against depth (Jeffery et al. 2017). Any allele showing a sigmoidal pattern with an exponential decay at each tail is considering a cline and 3) we will fit our allele frequency data using a Metropolis-Hasting algorithm as implemented in the hybrid zone R package HZAR using the functions *hzar.fitRequest* and *hzar.doFit* (Derryberry et al. 2014).

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**Figure 2.** Maximum likelihood cline shape expectation for loci under selection (left) and neutral (right). The two depths are reached within 150 m of linear distance with a maximum depth difference of 25 m between shallow and deep habitats.

**5. Intellectual Merit**

This project aims to examine the potential for reef restoration of a widely distributed an imperiled reef building coral on the Caribbean basin. By identifying the major genomic features that underlie coral adaptation to depth and testing whether the genomic bases of adaptation vary with geographic location, this study will directly measure fitness of adaptive alleles in the wild. Likewise, by quantifying the extent to which habitat-adapted populations can be used interchangeably across habitats, we can improve restoration of coral reefs. Our systematic assessment across depth gradients will not only provide a mechanism on how coral reefs maintain biodiversity through adaptive processes, but also deliver a plausible alternative to leverage urgently needed coral restoration efforts.

**6. Broader impacts**

With the severe decline of *O. faveolata* populations, recovery of this major Caribbean coral reef builder is paramount. Successful restoration depends on whether corals are able to thrive in transplanted sites by altering phenotypic traits, which often are underlined by genetic variation. The core of our proposal addresses a conservation issue with the goal of accelerating the recovery of this important Caribbean reef builder. In this project, we will build upon decades of study on coral adaptation to enhance restoration efforts of coral populations. Coral reefs need to be rapidly restored worldwide. Our efforts have served and will continue to serve the coral community to map genomic variation associated with climate change (Pinzón et al. 2015). We will continue to further develop beneficial coral genomic resources. Our genomic tools are publically available ([here](https://www.ncbi.nlm.nih.gov/Traces/wgs/?val=MZGG01#contigs)). For instance, other labs have used our reference genome to map 2d-RadSeq data (e.g. Matz – UT Austin, Meyer – Oregon State). Our study on the genomics of adaptation to depth in corals would bring scientists and professionals in coral farming together and implement cutting edge basic science findings into already established coral farms in Puerto Rico (NOAA) and the US Virgin Islands (The Nature Conservancy, TNC).

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