

## Project Narrative

The importance of coral reef ecosystems may be seen in their numerous ecological, aesthetic, economic and cultural functions (Maragos *et al.*, 1996). Not only is the coral reef structure itself composed of and built by, a diversity of organisms, but the reef structure serves as the basis for one of the highest diversity ecosystems in the world (Talbot, 1994). Florida's coral reef system is the third largest living reef on the planet and the only barrier reef system in the continental U.S. It underpins the state's marine ecosystems, provides habitat for over 6,000 marine species, attracts 16 million visitors per year, and in the Florida Keys alone supports over 70,000 local jobs, draws \$6.3 billion to Florida's economy (Gibson *et al.*, 2008; FL DEP, 2013). It also serves as the primary frontline of coastal resiliency defense from major storms. The Florida Keys, from Key West to Biscayne, have a 16% chance of experiencing the impacts of a hurricane in any given year. Hurricanes pass within 50 miles of this region every 6 to 8 years, and Key West (max elevation=18 ft) is directly impacted every 6 years, making this region highly susceptible to heavy flooding and storm surge during a hurricane event (e.g., Hurricane Irma resulted in high storm surge of over 4 ft in many areas). USGS has reported that a degraded coral reef leads to increased run-up in flooding. Observational and modeling studies indicate that had coral reefs been healthier across the Caribbean (including the Florida reef tract), the resulting wave-driven run-up and coastal flooding of areas fronted by coral reefs would have been less than did occur (due to the current degraded nature of the coral reefs).

Worldwide decline of coral reefs has been attributed to a variety of natural and anthropogenic stressors (Jackson *et al.* 2001; Harvell *et al.* 2002; Hughes *et al.* 2003; Bellwood *et al.* 2004), with coral species are facing severe threats from warming ocean waters, ocean acidification and disease. Coral habitats in the Keys have been in decline since the 1970s due to multiple stressors including coral bleaching, disease outbreaks, harmful algal blooms, hurricanes and anomalous weather patterns. Prior to coral decline, many reefs were dominated by the branching *Acropora* spp. and boulder corals, such as *Orbicella* and *Montastraea* spp. During the last 40 years, Florida's indigenous corals have declined in some areas by more than 90 percent, with some species losing more than 97 percent of their populations. NOAA recently announced new protections for coral with the listing of 20 new species as "threatened" — including several species found in the Florida Keys.

The environment plays a powerful role in dynamics of coral diseases. For example, warm water temperatures may increase pathogenic virulence (Ben-Haim *et al.* 2003), accelerate growth rates of pathogens (Remily and Richardson 2006), and compromise coral's immune system (Muller *et al.* 2008, Mydlarz *et al.* 2009). Populations of elkhorn and staghorn coral underwent a region-wide decline starting in the 1980s, with losses of up to 97% in some areas, mainly from increased prevalence of bleaching and disease. These two species were listed as threatened under the Endangered Species Act (ESA) in 2006. A more recent decline in the massive star corals have also led to a reduction in live coral cover in the Florida Keys (Ruzicka, 2013, Lirman *et al.* 2011). Great star coral (*Montastraea cavernosa*) and mountainous star coral (*Orbicella faveolata*), two important boulder coral species in the Florida Keys, were highly impacted by the 2010 cold water anomaly, particularly in nearshore environments. Some sites in the Lower Keys experienced more than a 95% decline in *O. faveolata* and a significant decline in *M. cavernosa*. As a result, the boulder star coral (*Orbicella annularis*), mountainous star coral (*O. faveolata*), and star coral (*Orbicella franksi*) are currently listed as endangered under the ESA.

Florida's coral reefs are currently experiencing a multi-year outbreak of a stony coral tissue loss disease (SCTLD). While disease outbreaks are not uncommon, this event is unique due to its large geographic range (hundreds of miles of reef tract), extended duration (4+ years), high rates of mortality (>90% on some reefs) and the number of species affected (>20 reef-building corals). The pathogen(s) has not yet been identified, but evidence suggests it may be transmitted by touch and through the water column, which makes it difficult to contain. The loss of reproductively active colonies, increased distance between sexually mature colonies and continuing spread of SCTLD have combined to create a situation in which it is unlikely that corals in the Florida Keys will repopulate the reefs naturally. Habitat protection,

conservation and threat abatement are likely not enough to stop the decline of reefs. Many agencies, scientists, and advisory groups (i.e., FKNMS's Coral Reef Ecosystem Restoration Working Group) have recommended the need for intensive coral reef restoration in the Florida Keys to address decades of coral loss. Restoration of the Florida Keys coral reefs, which are essential habitat for both commercial and recreational fisheries as well as the basis for a significant eco-tourism industry, will strengthen and expand this economic engine while concurrently addressing critical environmental conservation issues.

With the endorsement of elected officials at local, state and federal levels, Mote Marine Laboratory has proposed and begun to implement a strategic *Florida Keys Coral Disease Response & Restoration Initiative* in coordination and partnership with a consortium of coral research and restoration institutions and grass-roots organizations (i.e., the Florida Keys National Marine Sanctuary (FKNMS), Biscayne National Park (BNP), Florida Department of Environmental Protection (FDEP), Florida Fish and Wildlife Conservation Commission (FWC), The Nature Conservancy (TNC), Universities and Community Colleges, community volunteer groups and others). As a completely independent, nonprofit, global marine research institution that has existed for more than 60 years, Mote is ideally positioned to lead such an initiative as an institution having 1) demonstrated experience and expertise in developing innovative technologies for coral restoration, 2) significant existing coral research and restoration infrastructure and ongoing activities in the Florida Keys, 3) demonstrated ability to coordinate closely with appropriate federal and state agencies, as well as numerous partnering universities, and 4) demonstrated history of inclusion through significant local community engagement and outreach programs.

Mote has been studying coral ecosystems for decades and is an international leader in the development of innovative coral reef restoration technologies focused on growing threatened and reef building coral species for replanting on decimated or damaged sections of reefs throughout the Florida Keys in order to reverse decades of dramatic coral population decline. With ~\$7 million of philanthropic support, Mote has built a newly opened (May 2017) state-of-the-art marine science laboratory, *Elizabeth Moore International Center for Coral Reef Research & Restoration* (IC2R3), on its Summerland Key campus in the Florida Keys to serve as the base of operation for this restoration initiative. On an annual basis, over 100 researchers from more than 60 research institutions from around the world have partnered with Mote in utilizing its research infrastructure on Summerland Key. In the past decade, Mote scientists have planted more than 55,500 corals onto Florida's reefs, working with multiple partners to achieve effective restoration. In addition, the State of Florida has recently awarded a total of \$1 million for Mote, in coordination with FWC and FDEP, to significantly expand its science-based coral restoration initiative to plant an additional 38,000 reef-building and branching corals in the Florida Keys.

Through strategic utilization of transformative restoration technologies and selection of resilient coral genotypes, with attention to maintaining genetic diversity within and among species, scientists and local grass-roots volunteer groups are now poised to fully implement the bold and strategic *Florida Keys Coral Disease Response & Restoration Initiative* that Mote has envisioned with our partners. Our corals can't wait. We can continue to lament that it is impossible to bring back to life a dead 100-year old coral skeleton, as we continue to study and monitor coral reefs into extinction - or we can use science to respond to an ongoing epizootic ecological emergency and restore the Florida's coral reefs now.

Over the last decade, Mote has been a leader in coral restoration within the Florida Keys, outplanting tens of thousands of corals in an effort to jump start critical population recovery of several coral species threatened with extinction. Additionally, Mote scientists have been a critical contributor to the Florida Department of Environmental Protection's Disease Advisory Council efforts to understand and mitigate the current SCTLD outbreak, acting as the lead of the Restoration Trials Team Working Group. Financial support for Mote's coral reef research and restoration has primarily come from philanthropy with a range of \$100,000 - \$300,000 per year in support. This type of contribution from foundations and private entities will continue to contribute to Mote's coral reef research and restoration efforts. In addition, the State of Florida awarded a total of \$1 million dollars for Mote (FY 2017-2018 and

FY 2018-2019), in coordination with FWC and FDEP, to significantly expand its science-based coral restoration initiative to plant 38,000 reef-building and branching corals in the Florida Keys over that 2-year time span. The Florida State Legislature is currently considering an additional FY 2019-2020 award appropriation to continue this effort. A recent grant of nearly \$1.5 million from the National Fish & Wildlife Foundation (NFWF) will primarily support the research essential to quantify the resilience and resistance of each coral genotype to infectious disease exposure, increasing water temperature, and ocean acidification. Together, these funds total over \$1,000,000 per year in support to leverage in the implementation of the *Florida Keys Coral Disease Response & Restoration Initiative*. Our secured financial support includes funding to: conduct the essential genotyping of our restoration corals, identify coral genotypes resilient to climate change, ocean acidification and disease, initiate the establishment of a living coral gene bank, and conduct restoration up to April 2020. The present proposal would allow Mote to i) continue our coral restoration activities from January 2020 until December 2022, ii) quantify ecosystem function recovery of restoration sites, iii) increase the genetic diversity of corals used for restoration, and iv) double the size of an environmentally durable living coral gene bank and increase staff support.

With support from a NOAA Community-Based Restoration Program Coastal and Marine Habitat Restoration Cooperative Agreement, Mote and its partners will address critical gaps required to fully implement the urgently needed and strategic *Florida Keys Coral Disease Response & Restoration Initiative*. Specific annual activities of this proposal will include these 6 goals:

- 1) propagation of 8,800 corals through asexual fragmentation, raising of 1,200 sexual recruits, and outplanting of 10,000 “seedlings”<sup>1</sup> of diverse endemic coral species including genetic strains that have scientifically demonstrated high levels of resistance to coral disease along with resiliency to increased ocean water temperatures and/or decreased pH,
- 2) monitoring and assessing the survival of outplanted corals under baseline conditions and within the context of the stony coral tissue loss disease
- 3) assessing ecosystem recovery of coral restoration sites,
- 4) conducting necessary analytical research for identification of genetic resilience of endemic coral species with
- 5) targeting sexual cross-propagation of resilient genotypes to increase genetic diversity and resilience of the coral restoration propagation “pipeline”, and
- 6) implementation of coral disease response and restoration public education and outreach.

Mote’s innovative coral reef restoration technologies and the IC2R3 facilities will form the basis for implementing an urgently needed and strategic *Florida Keys Coral Disease Response & Restoration Initiative* in coordination and partnership with a number of other organizations. In doing so, this science-based coral restoration initiative will significantly address an ongoing ecological emergency by, *inter alia*:

- 1) furthering the adaptation of the Florida Keys coral reefs to changing conditions in Florida for the next 20 years and decades beyond;
- 2) restoring and expanding essential habitat for maintenance of diverse fisheries for commercial and recreational sustainable use;

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<sup>1</sup> ‘Seedlings’ is used as a term to represent either asexual fragments, including branching coral fragments and microfragments— see definition of each (in Goal 1 below), as well as new coral genotype recruits created from sexual propagation efforts.

- 3) implementing action to recover threatened and endangered coral species populations; and
- 4) increasing participation, support and practice in conservation stewardship among youth, community groups, and combat wounded veterans representing Florida's diverse population through direct engagement in Mote's volunteer citizen scientist coral restoration and conservation program.

### **Coral Restoration in the Florida Keys**

Coral restoration sites for this *Florida Keys Coral Disease Response & Restoration Initiative* will be located within areas of FKNMS and BNP (Figure 1). Mote scientists at IC2R3 have created and are currently using an innovative technology called micro-fragmenting, in which individual coral polyps are separated and grown at an accelerated rate for transplanting in the Florida Keys. This novel micro-fragmentation and re-skinning technique can, in essence, bring back to life massive brain, boulder, star and mounding coral structure that are vital for building reef substrate; in just two to three years instead of hundreds of years it might take nature to rebuild a coral on its own. The cutting-edge methodology illustrated in Figure 2 allows small fragments of brain, boulder and star coral to rapidly grow (Figure 3) and fuse back together (Figure 4 a & b) to form new coral tissue over the dead skeleton. Thousands of reef-building coral fragments are now being produced and replanted on depleted reefs in the Florida Keys – with great success (typical survival rates reach over 90% of most outplant sites).

In addition to restoring these more massive slow growing reef-building corals, science-based restoration advances with both the branching staghorn (*Acropora cervicornis*) and elkhorn (*Acropora palmata*) corals have led to thousands of genetically diverse fragments of the threatened corals being cultivated for restoration in extensive land-based and underwater offshore nursery sites (Figure 5) by Mote and a number of partner organizations in the Florida Keys. These branching corals are being used for replanting on decimated or damaged sections of the reef throughout the lower Florida Keys (Figure 6 and 7). Genetic identity of colonies has been established and nursery-grown staghorn coral grow four times faster than in the wild (Figure 8).

In 2014, FWC announced that staghorn corals grown in Mote's underwater nursery and later replanted by FWC researchers offshore of Marathon, FL had spawned in the wild — an exciting development demonstrating that corals grown with the innovative technology in a nursery setting and later used to restore a reef can reproduce naturally within only a couple of years. Recent evaluation of staghorn coral production techniques in an ocean-based nursery with consideration of coral genotype has contributed to a growing understanding of coral nursery culture, and will aid in the selection of genotypes for coral restoration. Mote and NOAA have also successfully increased survival of coral larvae from mass coral spawning events to produce thousands of one-year-old sexually produced coral recruits, instead of the one in a million that may survive in the wild. Together, these breakthroughs will allow large-scale nursery production of corals with higher genetic diversity and resiliency to disease and other stressors, as well as sexual reproduction of restored corals to help perpetuate biodiversity in the wild.

The work in this proposal will help support the Recovery of Acroporids, and implement actions in the Acropora Recovery Plan: <https://repository.library.noaa.gov/view/noaa/8950>. Note that there is no Recovery Plan for *Orbicella* sp., which is also listed as threatened under the ESA.

Actions addressed within the current proposal include:

Action 1: Implement Outreach and Education Strategies. See education and outreach goals.

Action 3: Conduct Strategic Research of Elkhorn and Staghorn Coral Biology. See resilience testing

Action 6: Conduct Active Population Enhancement. See restoration objectives.

Action 7: Understand Diseases affecting Elkhorn and Staghorn Coral. See resilience testing

Action 8: Respond to, control, and minimize effects of disease events. See resilience testing

Action 10: Develop and implement environmentally sound mechanisms to reduce local impacts of temperature stress. See resilience testing and sexual propagation

Action 11: Research and Develop Mechanisms to Enhance adaptation/acclimation of elkhorn and staghorn corals to increases in climate stress. See resilience testing and sexual propagation

Action 12: Restore, protect, and enhance ecosystem integrity and function. See ecosystem function monitoring goals.

**Goal 1:** propagation of 8,800 corals through asexual fragmentation and 1,200 sexual recruits, and outplanting of 10,000 seedlings

*Step 1: Expand in-situ (offshore) and ex-situ (land-based) coral nursery production in order to supply multiple coral species for research and restoration.*

*A. cervicornis* and *A. palmata* coral fragments are propagated in two existing *in-situ* coral nurseries using several previously established techniques, employing both materials deployed on the seafloor as well as structures suspended mid-water. Each method has benefits that are employed in order to facilitate different stages of the propagation process, as well as to spread the risk from various environmental factors that can impact corals growing within the nursery. *In-situ* propagation of nursery grown *A. cervicornis* and *A. palmata* will primarily utilize existing material already being maintained within established nurseries. However, additional wild collections of unattached *A. cervicornis* and *A. palmata* “fragments of opportunity” that have already broken from a wild parent colony and are found in circumstances that make their natural survivorship unlikely will occur from select donor sites in order to further increase the genetic diversity available in the nursery and for restoration efforts. Sexually reproduced corals from previous or upcoming spawning events will also be integrated into our established *in situ* nurseries to further increase genetic diversity. Nursery stocks are cared for year-round, with divers routinely cleaning the structures that hold the fragments during grow-out, and regular inspections of the anchoring systems that hold the nursery structures in place. Fragments are propagated during several periods each year, and an overall inventory by species and genotype is conducted quarterly in order to track the availability of fragments to support restoration and research needs. Currently, we have approximately 13,000 *Acropora cervicornis* and 350 *Acropora palmata* fragments within our in-situ nurseries (Table 1).

Mote’s land-based nursery, which propagates several species of corals for restoration and research (primarily focusing on *Acropora palmata*, *Orbicella faveolata*, *Montastraea cavernosa*, and *Pseudodiploria clivosa*; Table 1), produces coral fragments through both asexual fragmentation, as well as via sexual reproduction by settling larvae for eventual grow-out. Asexual propagation is accomplished by a newly developed technique known as “micro-fragmentation”. Most of the fragments currently being propagated using micro-fragmentation are from material already in-culture that originated from the FKNMS rescue nursery program which were previously transferred to the Mote land based nursery to be propagated in larger numbers. Coral “micro-fragments”, each approximately 1cm in diameter, are cut using a diamond edged band saw and then attached on to 3cm diameter ceramic plugs using gel super glue. Each individual plug receives a tag that identifies species and genotype number, which allows for us to inventory genotypes through time. Once mounted, micro-fragments grow at an accelerated rate in Mote’s outdoor raceways, quickly “sheeting” out over the ceramic plug in only a few months (Page et al. 2018). Once the fragment is covering approximately two thirds to the entire plug (3 cm diameter) it will be ready for a vet check and subsequent outplanting (see outplant ex-situ grown corals below). Although originally Mote used fragments of opportunity and upscaled a handful of genotypes for each coral species, a growing number of both *A. palmata* and *Orbicella faveolata* fragments originated from coral larvae that were collected in the field and settled in the laboratory. These sexually produced larvae originated from

different locations throughout the Florida Keys, including FKNMS and BNP. Over several years, these larvae grew into small colonies, which are now being integrated into our asexual reproduction efforts and are used to produce numerous replicate corals using the same micro-fragment techniques described above. Nursery stocks are cared for year-round by cleaning off algae and other growth inhibitors in order to maximize the accelerated growth of micro-fragments. Propagation of fragments in the land-based nursery also occurs year-round, and an overall inventory by species and genotype is maintained and updated every quarter in order to track the availability of fragments to support restoration and research needs. The inventory includes documenting the number of fragments by species and by genotype, and identifies the size class of each plug to better plan for upcoming outplanting activities. Currently, Mote has over 40,000 micro-fragments representing hundreds of genotypes of multiple branching, massive, and brain coral species.

**Table 1. Coral “seedlings” cared for in nursery by species**

| <b>Species</b>        | <b>In-situ</b> | <b>Ex-situ</b> |
|-----------------------|----------------|----------------|
| <i>A. palmata</i>     | 350            | 7,380          |
| <i>A. cervicornis</i> | 13,000         | 200            |
| <i>Montastraea</i> ,  | <b>0</b>       | <b>4,122</b>   |
| <i>Orbicella</i>      | <b>0</b>       | <b>11,264</b>  |
| <i>Pseudodiploria</i> | <b>0</b>       | <b>3,186</b>   |

*Step 2: Out-plant in-situ and ex-situ nursery grown corals to multiple degraded reef sites throughout the FKNMS.*

*Growing corals to outplant:* Once fragments grown through asexual propagation and located in the offshore nursery reach a suitable outplanting size, they will be collected and transported to nearby reefs for outplanting. Suitable sizes for outplanting are listed in Table 2.

*Outplanting Design (In situ raised Acropora cervicornis and A. palmata):* Once at the pre-determined restoration site, corals will be transplanted in a series of “arrays.” Each array will be comprised of multiple “plots” comprised of at least 5 genetically identical fragments arranged within a 0.5m diameter area, with approximately 1 - 2 m separating each plot (Figure 9 illustrates arrays and plots for *A. cervicornis*). While each plot contains one genotype and each array represents 5 plots of a single genotype, a series of arrays within a site will include a diverse set of genotypes to ensure genetic variability within an outplant site. The arrays will be oriented within close enough proximity to each other to maximize the potential for cross-fertilization during eventual broadcast spawning once colonies reach reproductive maturity. This configuration has been utilized in previous efforts in order to avoid dominance of any one genotype at each site and to maximize the diversity of genotypes from the available stock in our nursery.

*Outplanting Design (Ex situ raised Acropora palmata, Orbicella, Montastraea and Pseudodiploria):* Mote typically deploys 20 full-sized plugs for massive (*Orbicella*, *Montastraea*, *Pseudodiploria*) coral species and 5 full-sized plugs for branching (*Acropora palmata*) coral species within a single outplant array. This configuration for massive coral species provides enough initial coral surface area to create a single fused coral colony of ~25 cm within 2 – 3 years after outplanting (see Figure 2 and 4). The 5 full sized plugs for branching *A. palmata* will fuse within approximately 3 months and also reach reproductive size within 2 – 3 years. Mote has observed that a fused colony of this size in a laboratory setting is large enough to produce gametes and spawn, and Mote is currently in the process of more rigorously documenting this.

Table 2. Outplanting Summary

| Species               | Outplant Strategy              | Outplant Size (cm)                                 | # of corals to equal adult colony | # years anticipated to fuse to reproductive adult |
|-----------------------|--------------------------------|--|-----------------------------------|---|
| <i>A. cervicornis</i> | Arrays; nail and zip tie       | 25-50 cm TLE                                       | 5                                 | 2 - 3   |
| <i>A. palmata</i>     | Arrays; plug attachment, epoxy | 10-15 cm max. width (in situ) or 3 cm (land-based) | 5                                 | 2 - 3   |
| <i>Montastraea</i> ,  | Arrays; plug attachment, epoxy | 3 cm   | 20                                | 2 - 3   |
| <i>Orbicella</i>      | Arrays; plug attachment, epoxy | 3 cm   | 20                                | 2 - 3   |
| <i>Pseudodiploria</i> | Arrays; plug attachment, epoxy | 3 cm   | 20                                | 2 - 3   |

Outplant Method:

All coral outplants will be visually evaluated for health and land-based corals will be approved by certified veterinarian prior to out-planting. After a Release Authorization is issued by FWC for each health assessment on the land-based corals, coral outplants will be transported from the nursery to out-plant sites using previously demonstrated methods that ensure that corals are protected from any injury, direct sunlight, or additional stress, and will keep fragments from similar genotypes clearly identified. Mote will also finalize an outplanting implementation plan with NOAA prior to outplanting. The plan will include specifics on site selection, site locations, a design schematic showing outplant placement on the reef, procedures to minimize disease transmission, and the number and species of corals to be outplanted by location.

**Coral fragments** (*A. cervicornis*) will be securely fastened to the reef by attaching the fragments using cable ties to a small masonry nail that is driven into the bare substrate. Once secured, the *A. cervicornis* coral fragments quickly grow over the plastic cable ties, forming a new colony permanently affixed to the reef. Finally, a small tag imprinted with a unique identifier will be attached to the bare substrate directly adjacent to each plot using a 1" masonry nail, in order to permanently identify each plot of fragments for monitoring. Each array will be tagged with an identifier that denotes coral species and genotype outplanted within the array within a centralized database.

**Coral micro-fragments** (all other coral species) will have had sufficient time in the land-based nursery to establish on their mounting plugs. Micro-fragments are attached onto dead coral skeletons of similar species in arrays of multiple fragments of the same genotype by inserting the post of the coral plug into the hole drilled in the dead substrate, and using a small amount of epoxy or cement to attach the coral fragment and its associated ceramic plug flush to the surface of the coral skeleton. Each array will be tagged with an identifier that denotes coral species and genotype outplanted within the array within a

centralized database. Once secured, the coral fragments quickly grow over the ceramic plug, forming a new colony permanently affixed to the reef (see Figure 4).

*Outplanting site selection:* Selection will be highly region-specific and will include close coordination with local regulators and NOAA Restoration Center to ensure sites are appropriate prior to outplanting. Site selection criteria will include: presence of suitable reef habitat and/or historic presence of the species in recent decades. Out-planting events will also be distributed across multiple sites to minimize risk associated with changing environmental factors or impacts from storms or hurricanes at any one site. Additionally, *Acropora spp.* corals are believed to be resistant to the current epizootic so maintaining a random distribution of these sites within BNP and FKNMS is a scientifically logical approach to determine whether this hypothesis is supported with field data.

Out-planting will be distributed across multiple sites to minimize risk associated with changing environmental factors or impacts from storms or hurricanes at any one site. We will also use restoration activities to address critical questions related to the SCTLD outbreak, with guidance from FKNMS and FWC. When outplanting corals for determining disease resistance corals will be strategically out-planted within sites. The current epizootic is affecting primarily offshore reef sites within the lower Keys region. We will strategically select sites that span the offshore to inshore environment within this region. We will replicate genotype micro-fragment arrays within each site to determine which sites may be vulnerable to disease infection and which coral genotypes may be resistant to SCTLD. The disease has primarily moved through BNP, but current surveys suggest SCTLD will remain as an endemic within the region. We will out-plant corals within this region, again replicating sites within specific reef types and replicating genotype arrays to test for both endemic disease occurrence and genetic resistance within the out-planted corals.

The project lead will report the number of outplants by species across the restored sites at the end of the project.

## Goal 2. Monitoring Out-planted Corals

**Table 2. Tier I Monitoring\*\*\***

| <i>Metric</i>                         | <i>Sub-metrics</i> | <i>Project Target</i><br>(expected by end of project) | <i>Frequency of monitoring</i>         |
|---------------------------------------|--------------------|---|--|
| Percent Survival                      | Transplantings     | 80% (for all species)                                 | 1 month & 6-12 months post-restoration |
| Number of <i>Acropora cervicornis</i> | # Outplanted       | 6000 each year  | Counted as they are planted.           |
| Number of <i>Acropora palmata</i>     | # Outplanted       | 2800 each year  | Counted as they are planted.           |
| Number of <i>Montastraea</i>          | # Outplanted       | 400 each year   | Counted as they are planted.           |



|                                    |                        |   |                                |
|------------------------------------|------------------------|---|--------------------------------|
| Number of Orbicella                | # Outplanted           | 400 each year   | Counted as they are planted.   |
| Number of Pseudodiploria           | # Outplanted           | 200 each year   | Counted as they are planted.   |
| Number of additional coral species | # Outplanted           | 200 each year   | Counted as they are planted.   |
| Management Actions Implemented     | #                      | 8   | Reported in semi-annual report |
| Acres restored                     | #                      | ~10 acres per year  | Reported in semi-annual report |
| Community Enhancement              | Education and Outreach | Implemented (see Milestone Table and details below in Goal 6) | Reported in semi-annual report |
| Citizen Scientist Volunteers       | #                      | 2000 per year   | Reported in semi-annual report |
| Citizen Scientist Volunteers       | Hours                  | 8000 per year   | Reported in semi-annual report |

**\*\*\*\*Also see the Milestone Table which provides details associated with goals and actions funded by this award.**

Monitoring of outplanted branching corals will be conducted at 1 month and again between 6-12 months after outplanting (Table 2), as per monitoring requirements of our FKNMS permits and which surpasses expected Tier 1 monitoring associated with the present proposal. Outplants will be visually assessed by divers at all outplant location sites by a diver swimming around a site, relocating each array, collecting data on each array, and photographing the array with the tag visible. Outplants will be visually assessed by divers in order to monitor for survivorship, dislodgement, partial mortality, bleaching, disease, or predation. When possible, monitoring will be conducted on every outplant. Outplants will be monitored for the variables in the Table 3 and entered into Mote's Coral Restoration Monitoring Database. The number of acres restored utilizes guidance from the Acropora Recovery Plan with the goal that 5% of all suitable habitat will have approximately 25% coral cover. We estimate that approximately 5 Acroporid corals outplanted within a meter square will provide 25% coral cover within two years. We plan on outplanting a total of 8800 Acropora corals per year, covering a total of 1,760 meters squared. If this translates to a 5% of total habitat restored then the total acreage would be estimated as 8.5 acres. Similarly, we estimate that 20 plugs of massive coral species would equvalate to 25% coral coverage in 2 – 3 years. We estimate to outplant a total of 1200 plugs of massive corals species. Again, using the Acropora Recovery Plan estimate of 5% habitat at 25% coverage, this would equate to a total of 1.5 acres of restored reef with massive coral species each year. Therefore, we estimate the total acres restored per year would be approximately 10 acres. This method will be refined in consultation with the NOAA Restoration Center Outplanting Implementation Plan.

Table 3. Monitoring Plan and Metrics Summary

| Activity                  | Metric                            | Method                            | Frequency of Monitoring         |
|---------------------------|-----------------------------------|-----------------------------------|---------------------------------|
| Coral Restoration         | # Dead                            | Visual diver assessment           | At 1 month, between 6-12 months |
|                           | % Survivorship                    | Visual diver assessment           | At 1 month, between 6-12 months |
|                           | Breakage (yes/no?)                |                                   |                                 |
|                           | Partial mortality (yes/no?)       |                                   |                                 |
|                           | Bleaching (yes/no)                |                                   |                                 |
|                           | Disease (yes/no)                  |                                   |                                 |
|                           | Predation (yes/no)                |                                   |                                 |
| Microfragment Restoration | % Survivorship                    | Visual assessment & Photographs   | At 1 month, between 6-12 months |
|                           | Breakage (yes/no?)                |                                   |                                 |
|                           | Partial mortality (yes/no?)       |                                   |                                 |
|                           | Bleaching (yes/no)                |                                   |                                 |
|                           | Disease (yes/no)                  |                                   |                                 |
|                           | Predation (yes/no)                |                                   |                                 |
| SCTLD resistance testing  | Disease prevalence and resistance | Visual assessment and photography | Monthly                         |

**Coral Outplant Maintenance** of out-plant sites will include the removal of snails and fireworms from out-plants and reattaching broken branching fragments to the substrate. Any transplanted fragments that appear to be diseased and a threat to other coral colonies will be removed from the area, and a subsample will be preserved for health screening according to protocols established by FWC.

**Additional Stony Coral Tissue Loss Disease Epidemic and/or Invasion Zone monitoring** includes monitoring at 1 month intervals after out-planting for a total of 12 months. Higher frequency monitoring is needed to determine disease prevalence within a site and disease resistance among genotypes.

Methods: For all micro-fragments of susceptible coral species (*O. faveolata*, *M. cavernosa*, *P. clivosa*) that are outplanted to determine resistance to SCTLD, monitoring will be accomplished by photographing each tagged array of micro-fragments, allowing for detailed health assessment of each individual coral to be completed later by reviewing the photos. Data collected will be the same as that reported in Table 2. This method allows for a greater number of corals to be monitored at each monitoring event while still being able to capture individual differences between health and condition of each fragment in an array, to *verify if fragments fuse together*, and potentially obtain data on growth

over time. Disease activity, however, will be assessed *in-situ* as identifying disease-associated mortality is difficult from photographs.

Coral Outplant Maintenance (all coral species) of out-plant sites will include the removal of snails from out-plants and reattaching any dislodged microfragment plugs to the substrate. Any transplanted fragments that appear to be diseased and a threat to other coral colonies will be removed from the area, and a subsample will be preserved for health screening according to protocols established by FWC. This high level monitoring will far exceed the Tier 1 requirements of 90 days post restoration effort.

### **Goal 3. Assess the return of ecosystem function to restored reef areas (additional Tier 2 monitoring)**

RC Tier II Monitoring Question:

Did restoration actions change the reef community as demonstrated by increased number, diversity, and/or size of invertebrates (especially urchins), corals, or fish after 1 and 2 years?

Coral reefs are incredibly complex ecosystems, and species interactions can drive the dynamics and trajectory of these systems, especially once they have been disturbed. True restoration of resilient coral reefs therefore also must include restoration of ecological functions and relationships, such as plant herbivore interactions. Furthermore, restoration actions should ideally change the reef community by increasing the number, diversity, or size of invertebrates, corals, or fish. Therefore, coral restoration and monitoring activities will be accompanied by ecological monitoring that will occur at a six 10m x 10m offshore sites immediately before outplanting, and 12 months after outplanting (to give corals time to establish, and to control for seasonal effects). Criteria for sites to be selected include, but are not limited to:

- 1) Evidence of suitability for coral settlement and survival, as measured by high combined cover (>20%) of living and dead massive coral cover;
- 2) Presence of roughly equivalent coral cover to other experimental sites chosen for this project;
- 3) Presence of ledge habitat with bare rock suitable for *A. cervicornis* outplanting; and
- 4) Separation of at least 200m from other experimental sites used in this project.

Restoration Sites will be selected in collaboration with the NOAA Restoration Center prior to outplanting. Each restoration site will be paired with a similar, nearby 10m x 10m control site that will also be visited to ensure any changes can be attributed to restoration activities and not other factors. This Before, After, Control, Impact (BACI) design will be applied to all response variables measured. To assess ecosystem recovery, we will measure the following variables:

- 1) Coral cover, occurrence, and density (suggested by Ladd et al. 2019)
- 2) Coral diversity, species richness, and species evenness
- 3) Macroalgae and sponge cover
- 4) Fish biomass, abundance, diversity, and species richness
- 5) Benthic invertebrate (including coral) settlement
- 6) Herbivory rates and herbivore identity

Methods (Table 4)

**Variables 1-3** will be measured using a 1x1m grid method (for *Acropora spp.*) or by photogrammetry created with structure-from-motion using a GoPro hero 7 camera and Agisoft Metashape (previously photoscan) software following Young et al. 2017. This technique allows for 3D reconstruction of the

reef, providing considerable benefits over traditional 2-dimensional reef metrics. After images are used to construct 3D meshes, we will use meshes to quantify variables 1-3.

**Target:**

- For **variable 1**, our goal is to achieve 25% occurrence of *Acropora spp.* within each 10x10m site at a 1m scale. This is to say, each 10x10m site will be virtually divided into 100 1x1m squares, of which at least 25 will contain living *Acropora spp.* that did not before. As *Acropora spp.* colonies grow, this meets NOAA's Acropora Recovery Plan goal of 25% cover over 5% of suitable habitat while also addressing the challenges of assigning percent cover for branching corals. With respect to massive corals, substrate type interacts with other factors (such as sedimentation rate) to drive coral outplant survival (internal Mote unpublished data). As such, our goal for massive coral outplanting within our ecological assessment sites will be to re-skin 50% of deceased coral heads over 30cm in diameter to at least 30% cover per head after 12 months. Because of how massive coral outplants grow and fuse, this will eventually lead to near total cover of restored heads in subsequent years.
- **Variable 2** will be measured as a net increase in coral species richness at each site (i.e. number of coral species present as measured with photogrammetry), while maintaining coral species diversity (as measured by the Simpson species diversity index on a scale from 0 to 1) within 0.25 of initial diversity, which will vary by site. In this case, each distinct colony will be considered an individual for diversity analyses.
- **Variable 3** will be measured as a net decrease in percent algae cover and equal or greater percent sponge cover by month 12 when compared to pre-restoration, or when compared to non-restored sites in the same time step. Cover will be measured by applying a stratified random point count (five points for each meter squared) laid over the 3d photogrammetry mesh created for each site. Additionally, we will compare algae biomass loads on invertebrate settlement tiles in restored and non-restored sites as a secondary measure of relative algae loads.

To measure changes in coral associated fish communities (**variable 4**), we will use linear video transects. At each restoration and control site, we will establish 3 transects measuring 2m wide and the length of the restoration site. Transects will be run 3 times within month 1 and month 12 post restoration (n=9 transects per site per time period). Video will be analyzed onshore to get estimates of biomass, abundance, diversity, and species richness of teleost species at each site.

**Target:** Determined as in increase in fish biomass, abundance, and species richness, with species diversity remaining within 0.25 of initial estimates.

To measure benthic invertebrate settlement (**variable 5**), we will place benthic settlement tiles (n=12) at each restoration and control site every other month post-restoration, to ensure we capture invertebrate settlement, not merely invertebrate succession. Every other month, existing settlement tiles will be removed from each site and replaced with a fresh pair of tiles. Tiles will then be returned to shore for community analysis. Tiles will not be caged, to ensure dynamics incorporate all interactions that occur in the settlement process (i.e. predation).

**Target:** Same as variable 4, but will also include a net increase of coral settlement across all pooled restored sites when compared to non-restored sites.

We will measure herbivory by teleosts (**variable 6**), a dominant interaction in healthy coral reefs, with several food provision experiments to capture the roles of various herbivore guilds (i.e. browsers, grazers, etc.). To quantify top-down control by browsing herbivore teleosts, we will deploy 2 assays of 5 blades of the seagrass *Thalassia testudinum* both immediately before restoration and at 6 and 12 months post-restoration. To quantify top-down control by grazing and scraping fishes, we will also deploy a pair of tiles on which turf algae has been allowed to grow (tiles will be cultivated at Mote's IC2R3 facility on Summerland key). Primary producers will be weighed onshore prior to each assay. Assays will be left out for 2 hours, after which primary producers will be collected and re-weighed. Furthermore, we will record assays using GoPro cameras to record the identity of herbivores.

**Target:** Measured as a net increase in either (1) plant biomass removed or (2) rate of plant removal when compared to pre-restoration assays or contemporary assays at non-restored sites.

In all cases, we will analyze results in a BACI framework using generalized mixed effects models and model selection following Zuur et al. (2009) and Anderson (2007). To account for low site replication, models will be compared using AIC<sub>c</sub> (Symonds and Moussalli 2011). Benthic community variables in groups 1-3 (i.e. coral, sponge, macroalgae dynamics) will act as fixed predictor variables for ecological response variables (e.g. herbivory rates). Other variables (environmental variables, site identity, rugosity) will be included as random effects where appropriate.

Table 4. Ecosystem Function metrics, methods, targets and frequency of measurements

| Metric                 | Methods   | Targets   | Frequency                            |
|------------------------|---|---|--------------------------------------|
| Coral cover            | Complete photogrammetry of each 10x10m site       | At least 30% cover on at least 50% of dead coral heads by month 12  | Month 1, Month 12, yearly thereafter |
| Coral occurrence       | As above.   | 25% occurrence at 1x1m scale in restored sites by end of month 12   | As above.                            |
| Coral density          | As above.   | Net increase in coral density, as measured by number of colonies per 10x10m restoration plot and when compared to pre-restoration densities | As above.                            |
| Coral diversity        | As above.   |   | As above.                            |
| Coral species richness | As above.   | Equal or higher species richness when compared to pre-restoration richness, or when compared to non-restored contemporary sites             | As above.                            |
| Coral species evenness | As above.   |   | As above.                            |
| Fish biomass           | 3 transects per site, with 2 surveys per transect | Higher fish bio-mass across restored sites than non-restored sites. Higher biomass at restored sites in month 12 than pre-restoration.      | Every other month through month 12   |
| Fish abundance         | As above.   | As above.   | As above.                            |
| Fish species           | As above.   |   | As above.                            |

|                          |   |  |                                      |
|--------------------------|---|--|--------------------------------------|
| diversity                |   |  |                                      |
| Fish species richness    | As above.   | Higher fish species richness across restored sites than non-restored sites. Higher richness at restored sites in month 12 than pre-restoration.  | As above.                            |
| Invertebrate recruitment | Deployment of 4 benthic settlement tiles per site (benthic inverts); 3 transect surveys per site (urchins only) | Higher benthic invertebrate abundance, including coral recruit abundance as measured by settlement tiles. Higher benthic invertebrate species richness. Higher urchin abundance when compared to non-restored sites. | As above.                            |
| Herbivory rates          | Deploy 2 assays of <i>Thalassia testudinum</i> and 2 turf algae assays per site                                 | Net increase in absolute plant biomass removed, or in rate of removal.   | 0, 6, and 12 months post restoration |

### Monitoring costs

1-month (Tier 1) monitoring associated with outplanting *A. cervicornis* and *A. palmata*, either asexually propagated fragments grown within the in-water nursery, or microfragments sourced from the land-based nursery that originated from sexual recruits and later propagated through asexual fragmentation, will account for approximately 10% of the time for the four staff dedicated to the in-water nursery and outplanting effort (~\$28,000 per year, including salary, fringe and IDC). This effort includes not only the time in the field for data collection, but also data entry and management, and appropriate reporting. Monitoring efforts for massive corals will be led by the ecosystems postdoctoral researcher and the restoration staff, and will similarly account for approximately 10% of staff time (~\$22,000 per year, including salary, fringe and IDC). Additionally, initial dive surveys to find and assess candidate sites, set up data collection equipment, and quantify variables for ecological monitoring will require 40% of the postdoc staff time in month 1, immediately prior to outplanting activities (~\$4,000 per year, including salary, fringe and IDC).

1-year (Tier 2) monitoring associated with outplanting *A. cervicornis* and *A. palmata*, either asexually propagated fragments grown within the in-water nursery, or microfragments sourced from the land-based nursery that originated from sexual recruits and later propagated through asexual fragmentation, will account for approximately 10% of the time for the four staff dedicated to the in-water nursery and outplanting effort (~\$28,000 per year, including salary, fringe and IDC). This effort includes not only the time in the field for data collection, but also data entry and management, and appropriate reporting. Monitoring efforts for massive corals will be led by the ecosystems postdoctoral researcher and the restoration staff, and will similarly account for approximately 10% of staff time (~\$22,000 per year, including salary, fringe and IDC). Additionally, final dive surveys to quantify variables for ecological monitoring as well as to collect, enter, collate, and analyze data will require 60% of the postdoc staff time in month 12 (~\$6,000 per year, including salary, fringe and IDC).

Total per year of 1 month monitoring: ~\$54,000 with ~\$2,000 in supplies = ~\$56,000 per year

Total per year of 1 year monitoring and ecosystem function assessment: ~\$56,000 with ~\$15,000 in supplies = ~\$71,000 per year

Total: ~\$127,000 per year or ~13% of the budget

**Goal 4. Quantify the resilience and resistance of each coral genotype to infectious disease exposure, increasing water temperature, and ocean acidification and document in Mote coral trait database.**

Mote scientists at IC2R3 are using several techniques to propagate genetically diverse species of corals to restore basic ecosystem function within the Florida Keys coral reefs. To date, the branching corals have not exhibited susceptibility to the current SCTLD outbreak. However, white band disease decimated both *A. cervicornis* and *A. palmata* populations decades ago, and has become an endemic disease within the region. Research conducted by co-PI Muller has identified genetic strains of *A. cervicornis* that are resistant to white band disease (Muller et al. 2018). We are now studying the mechanism behind this resistance, screening other corals for these biological resistance indicators, and thoughtfully including this information within our restoration plan (see sexual reproduction research below). *We anticipate using these disease resistant corals within sexual reproduction events to simultaneously increase genetic diversity and disease resistance within our corals used for restoration.* Unfortunately, the majority of massive reef building coral species appear highly susceptible to the current SCTLD outbreak. Current efforts to stop SCTLD from progressing within infected corals using *in situ* treatments such as trenching and antibiotics has not been effective and are unattainable on the ecosystem scale. However, preliminary research suggests that several of the coral genotypes that Mote is using for restoration appear highly resistant to this current disease exposure (Figure 10), even after bleaching. Field based trials also support these results showing <1% disease prevalence of Mote's corals outplanted (n=2000 of species susceptible to SCTLD) within the invasion zone 4 months post-outplant. ***These results suggest that much of Mote's current and previous efforts to restore coral reefs will not be lost and that resilience-based restoration is the key for maintaining and restoring the coral reefs of the Florida Keys, especially within the context of SCTLD.***

In addition to the current devastating coral disease outbreak, Florida Keys corals will also continue to be impacted by warming ocean temperature and acidification (decreasing pH), likely for decades to come. In order for corals to survive 100 years from now, they will need to be resilient to all three of these stressors. Since corals are long lived, with no known limit on age, the corals we outplant today should ideally survive and thrive for hundreds of years. Therefore, it is important that we outplant corals able to survive our changing environment that also have an immune system resistant to known pathogens. Indeed, initial studies on *A. cervicornis* show that heat tolerance and disease resistance are independent from each other, and few coral genotypes may actually be resilient to both threats (Muller et al. 2018). Therefore, we acknowledge the critical need to maintain high genetic diversity within populations undergoing change, but understand that much of the out-planted corals need to contain resilient traits for future survival. As a result, we will utilize our resiliency information to conduct thoughtful applied restoration by out planting resilient corals, and increasing resilient traits within subsequent generations, while also maintaining high genetic diversity.

Funding for resilience testing is primarily supported by leverage funding from the National Fish and Wildlife Foundation for the first two years of the project. This leverage funding will be used to grow our resilient trait database, which will compare physiological responses to disease exposure, high water temperature, and ocean acidification conditions and the interactions of these threats among genotypes tested of the five main species of corals used for restoration: *Acropora cervicornis*, *A. palmata*, *Orbicella faveolata*, *Pseudodiploria clivosa*, and *Montastraea cavernosa*. Although other groups are starting to develop their own trait databases, Mote's efforts are unique because we are able to standardize environmental conditions within the laboratory setting and provide the replication needed to create robust results and interpretation of the phenotypic information by utilizing our state of the art Climate and Acidification Ocean Simulator. The present proposal directly funds the creation and care of the corals that we will use for these experiments.

**Proposed Methods:** Mote scientists will be exposing parent colonies of corals to different combinations of future ocean conditions for 2 months, followed by a suite of physiological and biometrics to quantify coral holobiont condition. After measurements are completed the corals will be exposed to

diseased corals from the field through direct physical contact between restoration corals and diseased coral and proximity non-direct physical contact exposure of diseased coral (i.e., sharing same water within same experimental chamber). At least five replicates of 5-10 putative genotypes of each species will be tested and quantified for resiliency traits in different environmental conditions each year. Buoyant weight, photochemical efficiency, photosynthesis and respiration rates, and calcification rates will be standardized to coral surface area and quantified for each replicate coral. Disease resistance will be quantified after 3 weeks of exposure. These trait values will be incorporated into the genotype database. Each biometric will be documented for each replicate coral. These data will be processed for important metrics and then incorporated into a final database, which will be used to compare resilience and resistance to the three most common threats to corals today, climate change, ocean acidification, and disease outbreaks. Highly resilient genotypes will be further screened to identify mechanisms underpinning these traits included genome analysis, gene expression (epigenetics), and microbiome characterization. **Outcome:** This data will be made freely available online and used to inform managers and practitioners to screen additional corals for these bio-indicators and to ultimately provide guidance for best management practices for restoration. Additionally, Mote corals are made available to collaborating researchers to request and use in subsequent experiments to further grow the resilience trait data base and further grow our knowledge base used for more effective restoration practices.

#### **Goal 5. Increase genetic diversity and resilience of the coral restoration propagation pipeline by conducting targeted sexual reproduction crosses during the spawning season**

Evolutionary response to potential future environmental challenges to coral reefs requires genetically-based variation among individuals. Therefore, maintaining genetic diversity within and between species is crucial for the promotion of resiliency, flexibility to adapt and long-term persistence through successful self-perpetuation of natural coral populations. Sexual reproduction is a mechanism crucial for generating genetic diversity. While increasing the gene pool of coral nurseries to be used for outplanting is one way of preserving genetic diversity, the inclusion of sexually-propagating corals within restoration strategies is paramount for the evolutionary potential and long-term survival of reef systems.

Therefore, the inclusion of sexual propagation can 1) generate the broad-scale genetic diversity necessary for natural populations to adapt to ongoing environmental changes, 2) generate novel genotypes, and thus phenotypes, relevant for adaptation to environmental stressors specifically plaguing the Florida coral reef tract, e.g. disease resistance, increased thermo-tolerance and increased skeletal density (to withstand intensifying natural disasters), and 3) provide access to thousands of propagules that can be used for experimentation (e.g. heritability analyses and assisted evolution; van Oppen et al. 2015, 2017), screening for other fitness-related traits (e.g. fecundity, calcification rates, rapid wound healing rates, bleaching resistance), and ultimately for inclusion into Mote's resilience-based restoration Initiative.

Within the present proposal, funding will assist with the addition of approximately **400 new coral** recruits a year for each of three species: *Acropora cervicornis*, *Acropora palmata* and *Orbicella faveolata*. A coral recruit will be considered a coral larva, created through sexual reproduction, that survives for 3 months after settlement occurs. Three months will be used a target date for success because that time will have surpassed initial mortality associated with fertilization, settlement, and post-settlement mortality. We will continue to grow and monitor survival of these settled corals until they transition into the asexual fragmentation pipeline of our land-based or in-situ nursery. After the transition, our nursery managers will continue to monitor their survival through propagation and outplanting. The size of these recruits at 3 months will vary depending on species but can range from 0.5 cm in diameter to several cm in diameter.

**Target:** 1,200 per year, 3,600 over the life of the project.



Mote is a leader in rearing and propagating corals from sexually produced coral spawn. Our previous collections include acquiring gametes from external sources such as Biscayne National Park, NOAA, the Coral Restoration Foundation and recently being successful in growing corals from cryopreserved sperm within a collaboration focusing on Assisted Gene Flow (in collaboration with Mary Hagadorn, SECORE, and CARMABI). Organizations have provided Mote with gametes because of our unique ability to properly settle and raise coral recruits from sexual propagation efforts; a task that eludes many other practitioners. To date, we have created thousands of new genotypes of *Acropora cervicornis*, *A. palmata*, and *Orbicella faveolata*. All of these acquired recruits are being integrated into our restoration pipelines. Although we will continue to acquire and collaborate with partners for sexual reproduction spawning events to increase genetic diversity, we will also target source populations of corals from the lower Keys, a region that has been neglected previously. We will use several sources of sexually-reproducing Lower Keys corals to increase genetic diversity and resilience within our restoration population.

*Outplanted populations:* Mote currently has ~7 different locations that can serve as *in-situ* source populations, 3 in Key West (Eastern Dry Rocks, Sand Key, Rock Key) and 4 near IC2R3 (American Shoals, Site U, Looe Key, Combat Wounded Vet Sites A, P, N). The number of genotypes currently included at each location and age (size) of corals varies across sites but one site, **Eastern Dry Rocks** (started in 2016), already has sexually mature colonies within its population. This site currently serves as the source population of gametes for co-PI Dr. Koch's research as IC2R3s visiting postdoctoral researcher. **Sand Key and Rock Key** (started in 2015) will likely be the next site to produce sexually mature corals, followed by the remaining younger sites (started 2018). Mote will use these outplanted populations to acquire gametes to create new genotypes that will feed into our restoration after growout occurs for one year within the lab. The *expectation of sexual maturity for these populations is 2-3 years, given they remain in optimal and un-fragmented conditions*. Co-PI Erich Bartel's *Acroporid* program continues to add more corals and genotypes across sites by utilizing these field collected sexual recruits. Funds from the present proposal will be used to conduct the gamete collections, crosses, and raising of these corals within the laboratory for future restoration use.

*In-situ spawning nurseries:* In April 2019, Dr. Koch created a spawning nursery in Mote's Looe Key *in-situ* grow-out nursery, comprising 10 genotypes (5 White Band Disease [WBD] resistant + 5 WBD susceptible, based on Muller et al. 2018). During summer 2019, a sexual maturity assessment will be conducted to determine sexual viability of each colony. Moving forward, we will add more genotypes to the nursery, and use this location as an alternative source for spawn collection for research. Focused sexual reproduction crosses of WBD susceptible and resistant genotypes will be conducted to determine whether subsequent generations are more resistant to WBD than control crosses, which would suggest that disease resistance is heritable and should be thoughtfully utilized within Mote's restoration plan. Again, the present proposal will fund activities associated with collection of field gametes acquired from these in-situ spawning nurseries. These corals will be grown and raised in the laboratory for ~ one year and then transitioned into a propagation and outplanting nursery (see *Outplanted populations* above)

*Ex-situ spawning induction system:* Using aquarium-trade equipment, artificial induction of coral sexual reproduction has been established by the Horniman Gardens and Museum in London (Craggs et al. 2017). This is a closed system mesocosm aquarium design that utilizes microprocessor technology to accurately replicate environmental conditions, including photoperiod, seasonal insolation, lunar cycles, and seasonal temperature for the purpose of inducing synchronized broadcast spawning *ex-situ*. **Funding from the current proposal** would **create one ex-situ spawning system at IC2R3**, within our already existing category 5 hurricane resistant building. Being able to induce spawning as needed has numerous advantages over using *in-situ* corals. First, one can induce more spawning events than what occur in nature (as low as once a year), providing many more opportunities for conducting experiments to answer specific research questions. Using a lab system provides more reliability, consistency and guarantee for spawn collection where inherent field risks like hurricanes and bad weather may prevent access to *in-situ*

corals during annual spawning events. Harvesting spawn from a tank indoors is considerably faster, easier and safer than doing so at night underwater. Using a lab system would remove logistical hurdles of having enough boats, captains, personnel to cover *in-situ* spawning events and, long-term, it should also cut down on costs associated with *in-situ* spawning, i.e. boat usage and field supplies. This system also opens the door for continued research on settling and rearing sexual recruits of species not currently well understood, many of which are highly susceptible to SCTLD, as well as give us more room to investigate genotypic incompatibilities, fertilization rates, fecundity and the dynamics of endosymbiont infection. Ultimately, an *ex-situ* sex induction system has the potential to propel sexual recruit, assisted evolution (i.e. selective breeding), genetic and restoration research forward, improving our chances of successful restoration of the coral reefs in the Florida Keys. Funding from the present proposal will assist with the establishment of this land-based sexual reproduction system (in Year 1 funding cycle) that will ensure vital collection of gametes during spawning season and year-round access to new recruits in the long term.

#### **Goal 6. Development of Coral Disease Response and Restoration Public Education and Outreach Initiative**

All education and outreach efforts are designed to promote teaching, learning and training leading to a greater understanding and ongoing discovery, and will include efforts focused on broadening participation of under-represented groups in scientific endeavors through strategic partnerships with existing community organizations, targeted recruitment. In addition to the 3 main focuses describe below and the numerous partnerships Mote has with education and outreach community groups and non-profit organizations, all of Mote's informal science education and public outreach facilities (i.e., Mote Aquarium on City Island, Sarasota, FL; Mote Coral Exploration Gallery at the Florida Keys History and Discovery Center, Islamorada, FL; and Mote Aquarium at Eco-Discovery Center in Key West, FL) that receive ~ 375,000 visitors per year will also highlight the activities and impacts of the Initiative.

There would be 3 main focuses of the education and outreach:

a) **Educational experiences for Florida Keys and visiting students** - Building on existing, evaluated programs at Mote Marine Laboratory, education staff will develop educational opportunities for upper elementary, middle and high school students throughout the Florida Keys region for hundreds of students annually, as well as visiting school groups from around the county. Programs will be developed for students to visit onsite at IC2R3, educators to visit local schools to deliver in-classroom lessons and live, interactive virtual programs to be delivered synchronously through Mote's Virtual Learning department. With an estimated 20 participants per class group, we will deliver educational programming to 800 school-age students per year for onsite experiences, 1,600 students annually with outreach into schools, and 400 students connected virtually. We would reach 8,400 students during the grant period.

These programs would be focused on increasing awareness around coral ecosystems, threats, and conservation and restoration efforts. Using educational content that is informed by the world-class research being conducted at Mote and partners, we will create interactive, relevant, and engaging learning experiences that are pedagogically appropriate to develop scientific, social, inquiry and critical thinking skills with the goal of promoting environmental stewardship and ocean conservation. Programs would be aligned with Florida state standards, as well as national Next Generation Science Standards.

Program outcomes will be assessed through formal and informal evaluation methods, including participant and teacher surveys, observations by program instructors and imbedded evaluative components (for example - repeat questioning in the beginning and end of the program to compare student responses). Select groups will be given pre and post surveys to compare content knowledge gains.

b) **Teacher professional development** – Mote Marine Laboratory will develop, deliver and evaluate a series of in-person professional development workshops for Florida K-12 educators focused on coral reef

ecosystems and associated topics, such as coral disease, ocean acidification, and current restoration and research efforts. Mote will host two workshops annually for up to 16 teachers each, for a total of 96 teachers over the grant period. The goal of these workshops is to increase awareness, understanding and communication of local impacts on the Florida Keys ecosystems. Educators will utilize Mote's and NOAA's data and tools, work with national and international coral reef researchers and access current research on coral ecosystems. Educators will be provided curriculum and classroom explorations to enhance K-12 students' science instruction related to this locally relevant ecosystem.

Using an established model utilized at Mote Marine Laboratory's City Island campus, these professional development workshops will combine scientific presentations, field experiences, participatory learning activities, laboratory demonstrations, facilitated discussions, data interpretation techniques, and guided access to science data. Each educator will receive locally relevant, age appropriate, science-rich lessons for their students incorporating best practices in instruction and effective communication techniques, aligned to state science standards.

Additionally, an online, asynchronous virtual teacher professional development workshop will be developed that can be accessed by teachers throughout the county. This initiative will capitalize on Mote's established, award-winning Education Digital Learning Department that delivers programs to between **3,000-5,000 people nationally each year**. This workshop will utilize the content and activities developed for the in-person workshop, but will be available on-demand for teachers to access remotely.

c) **Development of public citizen science coral outplanting program** - Mote will provide the opportunity for coral outplanting and monitoring by members of the local community, as well as visitors to the Florida Keys. In the first year, Mote will have thirty opportunities for citizen science volunteers to participate. Year two, we will expand our offerings and hold eighty annually and in year three we will host 8 per month for an annual total of 96. While the group sizes can vary, we will have an average of ten people per event, for over 2,000 total citizen science opportunities. Education staff will work with researchers to facilitate opportunities for students, teachers, and the public to participate in citizen science experiences to actively learn about and contribute to the ongoing research and restoration efforts, benefiting both the restoration ecosystems, as well as the participants. Based on proven models of citizen science, Mote will design a program that includes the necessary training through classroom-based instruction prior to in-water restoration and maintenance activities. The educational component of the citizen science program will be based on the aforementioned coral research and restoration activities, as well as incorporating citizen science volunteers in the actual work of the project. These activities will include SCUBA diving or snorkeling at established coral nurseries, conducting basic nursery maintenance, attaching fragments to in-water nursery structures, and assisting in outplanting nursery corals at local reef sites as part of ongoing coral restoration activities.

An online virtual experience will be developed which will allow those unable to participate a better understanding about the restoration process. Development and cultivation of strategic partnerships with community organizations and groups working with underrepresented audiences to provide citizen science opportunities will be a priority.

A full evaluation plan will be developed as part of the program design and development in the first quarter of the funding period. Evaluations will be implemented for each educational activity to monitor and evaluate outcomes, assess learning and outreach efforts and improve overall impact of the education efforts based on the anticipated outcomes including formative (both implementation and process evaluations) and summative evaluations. Summative reports will be created annually.

## MAJOR PROJECT OUTCOMES

Over a 3-year period, the *Florida Keys Coral Disease Response & Restoration Initiative* will significantly address an ongoing ecological emergency, further the long-term coastal resiliency adaptation

of the Florida Keys coral reefs to changing global conditions, and recovering threatened and endangered corals by a) restoring tens of thousands colonies of diverse endemic coral species and genotypes that are disease resistant, heat tolerant, and/or resilient to ocean acidification, b) expanding a rigorously standardized and robust resilient trait database (including disease exposure, high water temperature, and ocean acidification) and identifying mechanisms underpinning these traits, and c) providing freely available data to inform managers and restoration practitioners. The research experimental design on resilient coral genotypes will incorporate multiple threats and synergistic effects, include both asexual and sexual reproductive strategies, identify mechanism of resilience, and to scientifically evaluate and improve ecological impacts of this strategic restoration approach (see Figure 10 for entire Coral Restoration Cycle utilized by Mote Marine Laboratory).

Although not funded as part of this proposed cooperative agreement, Mote Marine Laboratory is part of a network of laboratories throughout Florida, providing facilities for living coral gene banks to preserve species, or particular genetic strains of species, that can be grown, propagated and transplanted back out onto the reef. Gene banks protect corals from natural disasters such as hurricanes, bleaching events and disease outbreaks. In fact, some genetic strains of corals now only exist within land-based coral gene banks. The 200-acre Mote Aquaculture Research Park (MAP) in Sarasota County, Florida, is an ideal location for the establishment of a new environmentally resilient U.S. national coral gene bank because it is located inland, experiences reduced hurricane activity and houses sophisticated recirculating seawater systems essential for maintaining marine life long-term. An initial procurement of funding from the National Fish and Wildlife Fund is jumpstarting the establishment of the Mote coral gene bank at MAP. However, additional funds are needed to expand and environmentally “harden” the infrastructure and operations; a need made clear from the coral communities’ effort to conduct the NOAA/FWC Coral Rescue Project, initiated by the FL DEP Disease Advisory Council’s Coral Rescue Working Group. During each year of the proposed cooperative agreement, Mote will seek to secure additional funding to leverage its existing state-of-the-art and patented recirculating seawater systems at MAP to increase both the coral biodiversity capacity and environmental resiliency of the coral gene bank infrastructure and operations with expanded redundancy of power supplies, as well as elevation and hardening of physical structures to potential environmental challenges.

## **PROJECT METRICS SUMMARY**

On an annual basis, we will propagate 8,800 corals through asexual fragmentation, raise 1,200 sexual coral recruits, and outplant 10,000 corals. A list of corals propagated and/or outplanted by species and number of fragments, along with monitoring data for out-planted corals, will be provided. Photos will also be provided to verify outplanted corals, as well as documentation of restoration sites before, during and after project completion. Summary data for routine inventory of number of corals in land-based and offshore nurseries will be submitted. The number of sexually produced recruits of each species will be reported. Results of experimental studies to quantify the resilience of each genetic coral strain tested will be provided. The trait based database quantifying each genotypes physiological response to disease, climate change and ocean acidification will be available for reference. The effects of restoration on ecosystem function will be assessed and provided within reports. Annual reports will be completed each year as well as the final report at the end of the funding period. Several peer-reviewed publications will be produced as part of this effort.

Table 5. Complete timeline of milestones and targets for tasks relevant to the current proposal including leverage funding tasks and type.

| Milestone  | Year 1 (January 1 - December 31, 2020) |      |      |      |   | Target   | Leverage Funding      | Leverage Type |
|--|--|------|------|------|---|--|-----------------------|---------------|
|  | Q1                                     | Q2   | Q3   | Q4   |   |  |                       |               |
| Goal 1. Step 1                                     |  |      |      |      |   |  |                       |               |
| Asexual propagation: A. cervicornis                | 400                                    | 400  | 400  | 400  | 1600  | 10,000 new corals propagated (in addition to NOAA RC effort)       | State of FL - secured |               |
| Asexual propagation: A. palmata                    | 1000                                   | 1000 | 1000 | 1000 | 4000  |  |                       |               |
| Asexual propagation: Orbicella faveolata           | 300                                    | 300  | 300  | 300  | 1200  |  |                       |               |
| Asexual propagation: Pseudodiploria clivosa        | 200                                    | 200  | 200  | 200  | 800   |  |                       |               |
| Asexual propagation: Montastraea cavernosa         | 300                                    | 300  | 300  | 300  | 1200  |  |                       |               |
| Conduct full nursery inventory (in situ)           | X                                      |      |      | X    | 15,000 corals   |  |                       |               |
| Conduct full nursery inventory (ex situ)           | X                                      |      |      | X    | 35,000 corals   |  |                       |               |
| Goal 1. Step 2                                     |  |      |      |      |   |  |                       |               |
| Outplanted: Acropora cerviconis                    | 1500                                   | 1500 | 1500 | 1500 | 6000  | 10,500 coral outplants   | State of FL - secured |               |
| Outplanted: Acropora palmata outplanted            | 700                                    | 700  | 700  | 700  | 2800  | 6,621 coral outplants  | RESTORE ACT - secured |               |
| Outplanted: Orbicella faveolata outplanted         | 100                                    | 100  | 100  | 100  | 400   | (in addition to NOAA   |                       |               |
| Outplanted: Pseudodiploria clivosa outplanted      | 0                                      | 100  | 0    | 100  | 200   | RC effort)   |                       |               |
| Outplanted: Montastraea cavernosa outplanted       | 100                                    | 100  | 100  | 100  | 400   |  |                       |               |
| Outplanted: Additional Species                     | 0                                      | 0    | 0    | 200  | 200   |  |                       |               |
| Goal 2.  |  |      |      |      |   |  |                       |               |
| Mote & NOAA final outplant implementation plan     | X                                      |      |      |      |   |  |                       |               |
| Monitoring of outplants                            | X                                      | X    | X    | X    | 80% survival  | monitoring outplants   | State of FL - secured |               |
| Goal 3.  |  |      |      |      |   |  |                       |               |
| Ecosystem Monitoring, pre-outplant                 | X                                      |      |      |      |   |  |                       |               |
| Ecosystem Monitoring, settlement tiles             |  | X    | X    |      |   |  |                       |               |
| Ecosystem Monitoring, post-outplant                |  |      |      | X    |   |  |                       |               |
| Goal 4.  |  |      |      |      |   |  |                       |               |
| Resilience Testing: A. cervicornis                 |  |      |      |      |   | Resilience Testing research (partial support)                      | NFWF-secured          |               |
| Resilience Testing: O. faveolata                   | X                                      | X    |      |      | 10 genotypes  |  |                       |               |
| Resilience Testing: A. palmata                     |  |      | X    | X    | 10 genotypes  | *note that no NOAA RC funds go directly towards resilience testing |                       |               |
| Resilience Testing: M. cavernosa                   |  |      |      |      |   |  |                       |               |
| Resilience Testing: P. clivosa                     |  |      |      |      |   |  |                       |               |
| Analyzing data                                     | X                                      | X    | X    | X    |   |  |                       |               |
| Expand coral trait database                        | X                                      | X    | X    | X    |   |  |                       |               |
| Goal 5.  |  |      |      |      |   |  |                       |               |
| Establish the indoor sexual induction system       | X                                      | X    | X    |      | Complete  | Sexual reproduction research (partial support)                     | State of FL - secured |               |
| Sexual recruits: Acropora palmata                  | NA                                     | NA   | NA   | 400  | 400   |  |                       |               |
| Sexual recruits: Acropora cervicornis              | NA                                     | NA   | NA   | 400  | 400   |  |                       |               |
| Sexual recruits: Orbicella faveolata               | NA                                     | NA   | NA   | 400  | 400   |  |                       |               |
| Increase genetic diversity of restoration pipeline | X                                      | X    | X    | X    | 10 new genotypes per species (50 new total genotypes) |  |                       |               |
| Goal 6.  |  |      |      |      |   |  |                       |               |
| Education activities evaluation plan developed     | X                                      |      |      |      |   |  |                       |               |
| Onsite education experiences for students          | 10                                     | 10   | 10   | 10   | 40 programs   |  |                       |               |
| Education outreach for Monroe County students      | 20                                     | 20   | 20   | 20   | 80 programs   |  |                       |               |
| Live, interactive virtual programs to students     | 5                                      | 5    | 5    | 5    | 20 programs   |  |                       |               |
| Teacher professional development                   | 1                                      |      |      | 1    | 2 programs  |  |                       |               |
| Citizen science monitoring and outreach trips      |  |      | 15   | 15   | 30 programs   |  |                       |               |
| NOAA RC reports                                    |  | X    |      | X    | 2 reports   |  |                       |               |
| Total Outplants                                    |  |      |      |      |   | 10,000   | 17,121                |               |
| Total Asexual fragments                            |  |      |      |      |   | 8,800  | 10,000                |               |
| Total Sexual recruits                              |  |      |      |      |   | 1,200  | TBD                   |               |

| Year 2 (January 1 - December 31, 2021)             |      |      |      |      |   |  |  |
|--|------|------|------|------|---|--|--|
| Milestone  | Q1   | Q2   | Q3   | Q4   | Target  | Leverage Funding   | Leverage Type                                      |
| Goal 1. Step 1.                                    |      |      |      |      |   |  |  |
| Asexual propagation: A. cervicornis                | 400  | 400  | 400  | 400  | 1600  | Additional propagates  | State of FL - not secured                          |
| Asexual propagation: A. palmata                    | 1000 | 1000 | 1000 | 1000 | 4000  |  |  |
| Asexual propagation: Orbicella faveolata           | 300  | 300  | 300  | 300  | 1200  |  |  |
| Asexual propagation: Pseudodiploria clivosa        | 200  | 200  | 200  | 200  | 800   |  |  |
| Asexual propagation: Montastraea cavernosa         | 300  | 300  | 300  | 300  | 1200  |  |  |
| Goal 1. Step 2.                                    |      |      |      |      |   |  |  |
| Outplanted: Acropora cervicornis                   | 1500 | 1500 | 1500 | 1500 | 6000  | Additional outplants<br>6,621 coral outplants<br>(in addition to NOAA<br>RC effort)  | State of FL - not secured<br>RESTORE ACT - secured |
| Outplanted: Acropora palmata outplanted            | 700  | 700  | 700  | 700  | 2800  |  |  |
| Outplanted: Orbicella faveolata outplanted         | 100  | 100  | 100  | 100  | 400   |  |  |
| Outplanted: Pseudodiploria clivosa outplanted      | 0    | 100  | 0    | 100  | 200   |  |  |
| Outplanted: Montastraea cavernosa outplanted       | 100  | 100  | 100  | 100  | 400   |  |  |
| Outplanted: Additional Species                     | 0    | 0    | 0    | 200  | 200   |  |  |
| Goal 2.  |      |      |      |      |   |  |  |
| Mote & NOAA final outplant implementation plan     |      |      |      |      |   |  |  |
| Monitoring of outplants                            | X    | X    | X    | X    | 80% survival  | monitoring outplants   | State of FL - secured                              |
| Goal 3.  |      |      |      |      |   |  |  |
| Ecosystem Monitoring, pre-outplant                 | X    |      |      |      |   |  |  |
| Ecosystem Monitoring, settlement tiles             |      | X    | X    |      |   |  |  |
| Ecosystem Monitoring, post-outplant                |      |      |      | X    |   |  |  |
| Goal 4.  |      |      |      |      |   |  |  |
| Resilience Testing: A. cervicornis                 | X    | X    |      |      | 10 genotypes  | Resilience Testing<br>research (partial support)<br><br>*note that no NOAA RC<br>funds go directly towards<br>resilience testing | NFWF-secured                                       |
| Resilience Testing: O. faveolata                   |      |      |      |      |   |  |  |
| Resilience Testing: A. palmata                     |      |      |      |      |   |  |  |
| Resilience Testing: M. cavernosa                   |      |      | X    | X    | 10 genotypes  |  |  |
| Resilience Testing: P. clivosa                     |      |      |      |      |   |  |  |
| Analyzing data                                     | X    | X    | X    | X    |   |  |  |
| Expand coral trait database                        | X    | X    | X    | X    |   |  |  |
| Goal 5.  |      |      |      |      |   |  |  |
| Establish the indoor sexual induction system       |      |      |      |      |   | Sexual reproduction<br>research (partial support)  | State of FL - not secured                          |
| Sexual recruits: Acropora palmata                  | NA   | NA   | NA   | 400  | 400   |  |  |
| Sexual recruits: Acropora cervicornis              | NA   | NA   | NA   | 400  | 400   |  |  |
| Sexual recruits: Orbicella faveolata               | NA   | NA   | NA   | 400  | 400   |  |  |
| Increase genetic diversity of restoration pipeline | X    | X    | X    | X    | 10 new genotypes per species (50 new total genotypes) |  |  |
| Goal 6.  |      |      |      |      |   |  |  |
| Education activities evaluation plan developed     |      |      |      |      |   |  |  |
| Onsite education experiences for students          | 10   | 10   | 10   | 10   | 40 programs   |  |  |
| Education outreach for Monroe County students      | 20   | 20   | 20   | 20   | 80 programs   |  |  |
| Live, interactive virtual programs to students     | 5    | 5    | 5    | 5    | 20 programs   |  |  |
| Teacher professional development                   | 1    |      |      | 1    | 2 programs  |  |  |
| Citizen science monitoring and outreach trips      | 20   | 20   | 20   | 20   | 80 programs   |  |  |
| NOAA RC reports                                    |      | X    |      | X    | 2 reports   |  |  |
| Total Outplants                                    |      |      |      |      | 10,000  | at least 6,621   |  |
| Total Asexual fragments                            |      |      |      |      | 8,800   | TBD  |  |
| Total Sexual recruits                              |      |      |      |      | 1,200   | TBD  |  |

| Year 3 (January 1 - December 31, 2022)               |      |      |      |      |   |                            |                           |
|--|------|------|------|------|---|----------------------------|---------------------------|
| Milestone  | Q1   | Q2   | Q3   | Q4   | Target  | Leverage Funding           | Leverage Type             |
| Goal 1. Step 1                                       |      |      |      |      |   |                            |                           |
| Asexual propagation: <i>A. cervicornis</i>           | 400  | 400  | 400  | 400  | 1600  | Additional propates        | State of FL - not secured |
| Asexual propagation: <i>A. palmata</i>               | 1000 | 1000 | 1000 | 1000 | 4000  |                            |                           |
| Asexual propagation: <i>Orbicella faveolata</i>      | 300  | 300  | 300  | 300  | 1200  |                            |                           |
| Asexual propagation: <i>Pseudodiploria clivosa</i>   | 200  | 200  | 200  | 200  | 800   |                            |                           |
| Asexual propagation: <i>Montastraea cavernosa</i>    | 300  | 300  | 300  | 300  | 1200  |                            |                           |
| Goal 1. Step 2                                       |      |      |      |      |   |                            |                           |
| Outplanted: <i>Acropora cervicornis</i>              | 1500 | 1500 | 1500 | 1500 | 6000  | Additional outplants       | State of FL - not secured |
| Outplanted: <i>Acropora palmata</i> outplanted       | 700  | 700  | 700  | 700  | 2800  |                            |                           |
| Outplanted: <i>Orbicella faveolata</i> outplanted    | 100  | 100  | 100  | 100  | 400   |                            |                           |
| Outplanted: <i>Pseudodiploria clivosa</i> outplanted | 0    | 100  | 0    | 100  | 200   |                            |                           |
| Outplanted: <i>Montastraea cavernosa</i> outplanted  | 100  | 100  | 100  | 100  | 400   |                            |                           |
| Outplanted: Additional Species                       | 0    | 0    | 0    | 200  | 200   |                            |                           |
| Goal 2.  |      |      |      |      |   |                            |                           |
| Mote & NOAA final outplant implementation plan       |      |      |      |      |   |                            |                           |
| Monitoring of outplants                              | X    | X    | X    | X    | 80% survival  | monitoring outplants       | State of FL - secured     |
| Goal 3.  |      |      |      |      |   |                            |                           |
| Ecosystem Monitoring, pre-outplant                   | X    |      |      |      |   |                            |                           |
| Ecosystem Monitoring, settlement tiles               |      | X    | X    |      |   |                            |                           |
| Ecosystem Monitoring, post-outplant                  |      |      |      | X    |   |                            |                           |
| Goal 4.  |      |      |      |      |   |                            |                           |
| Resilience Testing: <i>A. cervicornis</i>            |      |      |      |      |   | Resilience Testing         | NFWF-secured              |
| Resilience Testing: <i>O. faveolata</i>              |      |      |      |      |   | research (partial support) |                           |
| Resilience Testing: <i>A. palmata</i>                |      |      |      |      |   |                            |                           |
| Resilience Testing: <i>M. cavernosa</i>              |      |      |      |      |   |                            |                           |
| Resilience Testing: <i>P. clivosa</i>                | X    | X    |      |      | 10 genotypes  | *note that no NOAA RC      |                           |
| Analyzing data                                       | X    | X    | X    | X    |   | funds go directly towards  |                           |
| Expand coral trait database                          | X    | X    | X    | X    |   | resilience testing         |                           |
| Goal 5.  |      |      |      |      |   |                            |                           |
| Establish the indoor sexual induction system         |      |      |      |      |   | Sexual reproduction        | State of FL - not secured |
| Sexual recruits: <i>Acropora palmata</i>             | NA   | NA   | NA   | 400  | 400   | research (partial support) |                           |
| Sexual recruits: <i>Acropora cervicornis</i>         | NA   | NA   | NA   | 400  | 400   |                            |                           |
| Sexual recruits: <i>Orbicella faveolata</i>          | NA   | NA   | NA   | 400  | 400   |                            |                           |
| Increase genetic diversity of restoration pipeline   | X    | X    | X    | X    | 10 new genotypes per species (50 new total genotypes) |                            |                           |
| Goal 6.  |      |      |      |      |   |                            |                           |
| Education activities evaluation plan developed       |      |      |      |      |   |                            |                           |
| Onsite education experiences for students            | 10   | 10   | 10   | 10   | 40 programs   |                            |                           |
| Education outreach for Monroe County students        | 20   | 20   | 20   | 20   | 80 programs   |                            |                           |
| Live, interactive virtual programs to students       | 5    | 5    | 5    | 5    | 20 programs   |                            |                           |
| Teacher professional development                     | 1    |      |      | 1    | 2 programs  |                            |                           |
| Citizen science monitoring and outreach trips        | 24   | 24   | 24   | 24   | 96 programs   |                            |                           |
| NOAA RC reports                                      |      | X    |      | X    | 2 reports   |                            |                           |
| Total Outplants                                      |      |      |      |      | 10,000  | TBD                        |                           |
| Total Asexual fragments                              |      |      |      |      | 8,800   | TBD                        |                           |
| Total Sexual recruits                                |      |      |      |      | 1,200   | TBD                        |                           |

## PROJECT TEAM

- Dr. Crosby (PI) has over 35 years of leadership and management experience in diverse, trans-discipline, multi-institutional national and international research initiatives with multiple universities, national and international science and resource management agencies, programs, and committees. As a scientist, Dr. Crosby has developed successful research grants of more than \$30 million, such as a U.S. Man and the Biosphere Program project entitled "Ecological and socio-economic impacts of alternative access management strategies in marine and coastal protected areas"; the U.S., Israeli and Jordanian joint partnership project entitled "The Red Sea Marine Peace Park Cooperative Research, Monitoring and

Management Program”; and the Hawai‘i State EPSCoR project entitled “Pacific High Island Evolutionary Biogeography: Impacts of Invasive Species, Anthropogenic Activity and Climate Change on Hawaiian Focal Species.” He is a Fellow of the Linnean Society of London, served 12 years on the Board of Governors and was past Chairman for the U.S.-Israeli Binational Science Foundation, is a Past-President for Sigma Xi-The Scientific Research Society, and is currently on the Board of Directors (Past-President) for the Association of Marine Laboratories of the Caribbean, Board of Directors (Past-President) for the Pacific Congress on Marine Science and Technology, Executive Committee Chairman for the Florida Institute of Oceanography, and Board of Directors Chairman for the Southeast Coastal Ocean Observation Regional Association. He serves as a reviewer and panelist for numerous scientific journals and national and international science panels and advisory committees, and has published more than 50 articles in journals, technical memoranda series, and has edited several books and manuals dealing with marine protected areas and coral reefs. With this experience and his position as Mote President & CEO, Dr. Crosby is ideally positioned to lead, provide oversight for, and ensure the success of a Coral Disease Response & Restoration Initiative in collaboration with numerous partner organizations.

- Dr. Muller (co-PI) is Science Director of Mote’s Elizabeth Moore International Center for Coral Reef Research & Restoration (IC2R3) and Manager of the Coral Health and Disease Research Program. She is an NSF Early Career Scientist, studying coral health and disease dynamics with a comprehensive research approach spanning from understanding shifts in microbial assemblages of corals, to applying hierarchical spatial and temporal models for understanding the drivers causing coral-disease outbreaks, and variability in resilience amongst and between corals species and genotypes. Dr. Muller is also a recognized expert and active participant in ongoing federal, state, and inter-institutional Florida Disease Advisory Council working groups, is the lead of the Restoration Trials Team and will work closely with Dr. Crosby to facilitate overall coordination between all aspects of the Coral Disease Response & Restoration Initiative.
- Erich Bartels (co-PI) is Mote Staff Scientist and Manager of the Coral Reef Monitoring and Assessment Program. Based at IC2R3 in Summerland Key, Florida, Bartels has worked on projects related to coral reef research, monitoring, and restoration for the past 20 years. He will lead all field out-planting aspects for coral restoration, is a primary contact with federal and state agencies for coral restoration permits, and coordinates all marine operations related to visiting and resident researcher studies on coral research and restoration.
- Dr. Emily Hall (co-PI) is Mote Staff Scientist and Manager of the Ocean Acidification Research Program, with over 10 years of marine and coastal chemical ecology research experience. She will coordinate NFWF research associated with ocean acidification flow-through experimental raceway units, provide expertise associated with lab and mesocosm coral exposure studies, and collaborate in coral holobiont research.
- Dr. Robert Nowicki (co-PI) is a Mote Postdoctoral Research Fellow with over 6 years of experience working in degraded marine ecosystems. As a community and disturbance ecologist, he has experience tracking the impact of resource and habitat loss on fauna communities, as well as determining how ecosystem engineers recover over time and how species interactions alter recovery patterns. He will lead efforts to monitor and quantify changes to community and ecosystem parameters in response to restoration efforts.
- Dr. Hanna Koch (co-PI): is a visiting postdoctoral fellow supported by the German Research Foundation. Dr. Koch’s research interests cover a broad spectrum within marine and evolutionary ecology, including adaptive evolution. Generally, her research focuses on studying how organisms cope with environmental change, whether that change stems from abiotic conditions linked to climate change or biotic factors related to species interaction. The focus of her work will be to use sexually-reproducing corals for studying which genetically-based traits may provide threatened coral species with increased resistance or resilience to environmental stressors linked to climate change, and then use this information to advance coral restoration strategies.



- Aly Busse (co-PI): is Mote's Vice President of Education. She comes from a diverse background in informal science education, including aquariums, museums and community outreach programs. Before joining Mote Marine Laboratory, Aly was Education Director at UnderWater World, Guam and Youth and Family Programs Coordinator at the Pacific Science Center. She also held dual roles at Rutgers University. She was the Senior Program Coordinator for a mobile science outreach program connecting STEM graduate students with middle schools around New Jersey through interactive science programs and served as Associate Director of the Rutgers Geology Museum, developing and implementing new school and public programs. In her past and current position, she works to bring scientists and their research to the public, translating current scientific findings into meaningful, interactive opportunities for a variety of audiences.

**Amount of Budget spent on building Ecosystem Response and Resilience to Disease Outbreaks:** arguably 100% of the budget is going towards the response to stony coral tissue loss disease. The proposal targets identifying corals resistant to disease and more likely to survive this outbreak and future outbreaks to come. Indeed, it is crucial that we incorporate resistant corals within coral restoration so that recovery can occur while this outbreak, and other major global threats, continues to affect coral reefs of the FL Keys. Additionally, coral restoration utilizing resistant genetic strains of endemic coral is the only way to recover coral reef ecosystem function after decades of decline throughout the Florida Reef Tract. Although protection and management have made great strides in reducing point-source, non-point source and other direct human impacts, no population recovery has occurred for reefs of this region. Coral restoration, therefore, is a direct and targeted response to the current outbreak, and other major threats that have depleted our coral reef ecosystem.

#### Year-1

- 1) conducting necessary analytical research for identification of genetic resilience of diverse endemic coral species and targeted cross-propagation of resilient genotypes;
- 2) propagate 8,800 corals through asexual fragmentation, raise 1,200 sexual produced coral recruits, and outplant 10,000 corals consisting of diverse endemic coral species, integrating genetic strains that have scientifically demonstrated resiliency to coral disease, increased ocean water temperatures, and/or decreased pH;
- 3) initiate multi-year monitoring component to scientifically evaluate and improve impacts of restoration;
- 4) Establish baseline information to quantify the return of ecosystem function of restoration sites compared with those left to 'recover' without assistance;
- 5) Establish several sources of coral gametes to increase genetic diversity and resilience within our restoration population

#### Year-2 and Year-3 (each)

- 1) Continue to conduct necessary analytical research for identification of genetic resilience of diverse endemic coral species and targeted cross-propagation of resilient genotypes and establish mechanisms for resilience;
- 2) propagate 8,800 corals through asexual fragmentation, raise 1,200 sexual produced coral recruits, and outplant 10,000 corals consisting of diverse endemic coral species, integrating genetic strains that have scientifically demonstrated resiliency to coral disease, increased ocean water temperatures, and/or decreased pH;

- 3) continue to conduct the multi-year monitoring component to scientifically evaluate and improve impacts of restoration;
- 4) Quantify the return of ecosystem function of restoration sites compared with those left to ‘recover’ without assistance;
- 5) Collect gametes from several sources of sexually reproductive corals to increase genetic diversity and resilience within our restoration population

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