Acropora Recovery Implementation Team
Working Group Report and Recommendations on Genetic Banking
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Background:

The Acropora Recovery Implementation Team (ARIT) has identified genetic banking (recovery action #6d) as a priority action for *Acropora* recovery due to 1) the realized potential for rapid loss of genotypes in the wild population (e.g. range-wide bleaching and hurricane impacts between 2014-2017), 2) the burgeoning interest in novel interventions for bolstering coral resilience that could utilize such banked resources, and 3) the potential for the ARIT to play a constructive role in moving this action forward. The ARIT Genetic Banking Working Group has been convened to assist in progressing this action. The focus of the group has been on cryobanking, though the group membership incorporated participants in both land-based and *in situ* nursery banking and our recommendations may be appropriate for both.

The goals of the working group were to devise a comprehensive strategy for cryobanking to provide genetic resources for *Acropora palmata* and *A. cervicornis* in order to enhance recovery prospects. We chose to focus on sperm collections in the current recommendations as these provide the best-developed cryotechnology for corals (Hagedorn et al. 2012, 2017; Daly et al. 2018) and the broadest utility, but presumably the principles of such a plan could be easily expanded to address additional sample types (e.g., cryopreserved oocytes, larvae or whole adult tissue). We also anticipate that these recommendations could be applied to other coral species and to designing live bank collections. Additionally, the Working Group acknowledges the obligate nature of symbionts in the viability of corals, making them perhaps unique in genetic archiving targets. Hence genetic resources for coral symbionts strongly warrant consideration but are beyond the scope of the current plan (though some additional considerations are given in Appendix 1). Clearly, to the extent that whole-tissue samples can be included in cryo-archives, this has the added benefit of preserving the entire holobiont. However, at the current time, reliable protocols are not yet available for effective cryopreservation of mature coral tissue, but expected to be available within a year (M. Hagedorn, pers. comm.).

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Goals of Banking Genetic Resources:

Within the context of the *Acropora* Recovery Plan, the ARIT Genetic Banking Working Group identified the following goals for genetic banking efforts. It is acknowledged that these goals are likely served via qualitatively different collection management schemes (see section below).

1) Curate and maintain a genetic repository of samples which represent the range of extant, genetically distinct individuals, thus capturing genetic as well as allelic diversity. Such an archive should provide genetic potential for re-colonizing extirpated populations as a last resort. It is noted that in this 'worst case' situation, even representative and high quality sperm collections are probably inadequate. Such an endeavor would likely require live-banked material and/or larvae to reconstitute a viable population.

2) Provide genetic diversity for the development of techniques such as assisted gene flow or selective breeding to enhance the genetic diversity and potential viability of existing local (or captive breeding) populations

3) Provide genetic resources for research, especially to better characterize potentially adaptive traits and unforeseen future needs.

Recommended Banking Structure and Usage Guidelines:

The intent of this strategy is to provide for a public, centralized (i.e., few and closely coordinated locations), multi-purpose bank of genetic resources to facilitate ESA recovery. Given the hurdles in transferring genetic resources internationally (including CITES and the Nagoya Protocol of the Convention on Biological Diversity), it is understood that samples may need to be archived within the country from which they were collected. This plan includes recommendations for range-wide collection, and we encourage international coordination in managing genetic resources. However, we recognize that for practical reasons, US colonies may be targeted for initial contributions to an ESA-recovery focused archive. For US collections, and any specimens that are importable into the US, the US Dept of Agriculture National Animal Germplasm Program has, in principle, capacity and authority to access and archive an ESA *Acropora* genetic bank as described. However, recent disease outbreaks in the Caribbean region, though not affecting *Acropora* spp. directly, raise the likelihood that separate, quarantined tanks would be needed for any samples being collected/archived from current Caribbean collections. Further discussions between NOAA/NMFS/Office of Protected Resources and USDA to clarify responsibilities in such an inter-agency endeavor are needed. Presumably, the specifications of ownership and material transfer agreements will be influenced by the policies of the particular institution operating the storage facility, but for the purposes of ESA recovery, there should be a bias for public ownership and access. In addition to the described sperm banking, genetic vouchers (likely tissue biopsies with appropriate DNA fixative such as ethanol) to facilitate genotyping of donor colonies for which genetic information is lacking are also required; this voucher collection may be archived in a separate institution (e.g., a museum such as the Smithsonian or other archival institution[2]) as these samples are intended to be destructively analyzed (for genotyping) when resources allow. It would be important to establish a dedicated curator to coordinate the collection, archiving, accession, data

[2] In very preliminary inquiry, the Ocean Genome Legacy Center at Northeastern Univ had expressed interest in serving this archival function for the genetic voucher collection

management, and withdrawals of both sperm and genetic voucher specimens, including among international partners.

Given the diverse goals associated with genetic banking, it is anticipated that the sperm bank should be partitioned into separate collections addressing different goals. We suggest that a 'Vault' collection be archived in perpetuity, specifically as 'insurance' addressing Goal 1 - a most dire situation which we hope to avoid. Goals 2 and 3 are viewed by the Working Group as priorities in the near term and require more flexible archives that are regularly accessed for research, development, and testing of novel interventions. Ballou et al. (unpubl.) analogize this type of collection to a 'Checking Account'. Ideally, as collections are made, an adequate volume of material can be collected to populate both of these reserves. In situations where adequate material is not available for both, strategic decisions will need to be made in allocating between the different bank types; dedicated curatorial staff would be a key component of such collection management.

Generally, samples in the 'Vault'' collection are not anticipated to be used except in emergency situations (near, but prior to, extinction or broad geographic extirpation). However, it is anticipated that the 'Checking Account' collection is to be used regularly for approved research and enhancement activities. We anticipate that applications may be made by researchers or conservation practitioners/managers to access samples in this collection. It is difficult to anticipate all of the appropriate use cases that are likely to emerge in this rapidly evolving field. Hence, we suggest that a standing advisory board be formed to evaluate such requests in light of available holdings and current and future research and management priorities (e.g. National Academies of Sciences, Engineering, and Medicine 2019). It is also suggested that such requests be explicitly evaluated in terms of the minimum sample quality that will meet the intended purpose (e.g. genomic analysis versus crosses to develop viable larvae), as it is possible that, despite appropriate quality control efforts and screens, low quality sperm samples will be present in the bank (see discussion below).

Collection Strategy

This Working Group reviewed literature on existing plant and animal genetic banking guidelines and population genetics for the two species in question. An optimal collection strategy depends on predefined targets (e.g. in terms of extant genetic variation captured in the collection) and, ultimately, on the goals and intended uses for the bank. The Working Group adopted the following targets and usage goals in defining the recommended collection strategy:

*Collection Target*: To the extent practicable, we recommend maximizing the allelic richness of the collection to ensure common, rare, and potentially adaptive alleles are represented. Recognizing that access to (and therefore the cost to acquire) high quality sperm samples is likely to be limiting, the recommendations in this plan focus on a hierarchical approach. Geographic patterns of genetic variation in the target species are relatively well-documented (see reference list on population genetics) and more information is likely to become available in the next few years as a full scale SNP panel (~40,000 loci; Kitchen et al. 2019, Baums, pers. comm.) and other cost-effective genetic assays become available. It seems unlikely that major changes in the current understanding of patterns of genetic structure will occur with the

additional resolution of the enhanced SNP data, so the higher geographic levels of the hierarchy should not change.

Based on various genetic studies, the following 'genetic regions' are proposed:

- Florida (multiple sub-regions probably make sense based on Drury et al. 2017)
- Bahamas (latitudinal sub regions probably make sense)
- USVI/Puerto Rico
- Meso-America (Mexico [Caribbean and Gulf of Mexico coasts], Belize, Honduras, Panama shown distinct from Florida in various studies)
- Southeast/Lesser Antilles (most data from Curaçao, but likely useful subregions throughout)

Within genetic regions, the goal is to sample from replicated sites stratified among diverse habitat types at each of several locations/subregions, and particularly from habitats/sites where the presence of the species is unexpected. Colonies growing in unexpected environments may possess unique allelic variants. Local populations of corals likely display the greatest genetic variance across gradients of depth, cross-shore habitat zone, turbidity (affecting light and probably particulate organic carbon and nutrient concentration), and daily temperature range, so these are important habitat clines from which to sample.

*Suggested Habitat Stratification*: We suggest sampling from at least two reef sites for each habitat stratum (but including all 'extreme' habitats) per location (or sub-region) within each genetic region, with the understanding that the species may not be present in all of these habitats in all locations. Likely habitat strata for *A. palmata* and/or *A. cervicornis* include:

-Near shore/lagoonal patch reefs (2-4m depth; more turbid, more temp fluctuation)
-Shallow back reef (<2m depth)
-Shallow crest/fore reef (2-8m depth)
-Deeper forereef (>10m; may not be present in all locations)
-Extreme Habitats: including but not limited to urban seawalls, intertidal crests, very deep (>20m), areas of high temperature fluctuation.

We recommend targeting a minimum of 10 distinct genotypes from each of two sites yielding 20 genotypes per habitat stratum per location; see Appendix 2 for hypothetical examples). This number is somewhat arbitrary, but would encompass collections across duplicated sites in multiple habitat strata such that at least 40-50 distinct genotypes would be represented from each genetic subregion. These numbers would meet both the theoretical 'rule of thumb' targets (50 randomly selected individuals per 50 'populations'; Brown and Marshall 1995) and the targets of Shearer et al. (2009) based on coral microsatellite rarefaction analyses. Both target species can be highly clonal (i.e. many nearby colonies may represent clones of each other and thereby only a single genotype). If *a priori* knowledge of clonal structure is not available for a given site the chances of inadvertently collecting and archiving multiple redundant samples should be minimized by targeting colonies >5m distant from each other, avoiding 'thickets',

which are more likely to be clonal, and encompassing any obvious morphological variants. Drury et al. 2016 show high nucleotide diversity among colonies within locations for *A. cervicornis*, so this increases confidence that haphazard collections can be effective.

However, this strategy becomes challenging in the context of sperm sample collections, as the target species often manifest troubling levels of asynchrony in their spawning, and spawning night can be genotype-dependent. This often leaves collectors with little choice of parent(s) from whom collection of sperm samples is feasible on a given night. Nonetheless, efforts should be made to disperse these samples spatially and to tag colonies from which sperm samples are collected in order to obtain, at a minimum, **GPS location of the parent colony and a biopsy sample to serve as a genetic voucher for future genotyping**. Similarly, 10 distinct genotypes may not be present within many individual dive sites. In such cases, genotypes should be collected from additional sites up to the target of 10 from that location/habitat stratum. **Samples should not be collected/archived from colonies with active disease signs**.

As the bank is built, it would likely be beneficial to perform subsetting analyses to test the actual, realized, allelic characteristics of the bank (or subsets of the full banked collection) against whatever range-wide species pop-gen databases are available. Such analyses could provide a means to improve the efficiency of the collection strategy (e.g. indicate that more or fewer genotypes are needed from particular strata)(Hoban et al. 2018)

Practical Considerations in Sperm Collection

*Individual versus Pooled Samples*:

Ideally, samples from each individual colony would be archived separately to maximize the resolution of potential future usage (e.g. as particular functional genetic traits are resolved, they could be applied in selective breeding). However, realistically, time and resources are extremely limited during spawning events, and there may be opportunity to preserve more (and more diverse) material by combining sperm collected from multiple colonies in pooled sperm samples. Overall, we suggest prioritizing effort toward archiving samples from individuals, but also including pooled samples if this increases the total allelic diversity preserved.

*Quantity and Quality of Samples:*

Given the wide range of potential uses for samples in this genetic archive, it is advised that the largest feasible quantities of sperm for each sample be archived. For example, a single feasibility study for implementing assisted gene flow in *A. palmata* conducted in 2018 (Hagedorn et al. 2018) depleted the current national *A. palmata* sperm bank by 20%. There is also evidence of low sperm quality in many current-day coral sperm collections and there are likely substantial opportunity costs to archiving samples that will not be reasonably viable. Thus, basic measures of pre-freeze and post-thaw sperm motility must be assessed and provided with the metadata record for each sample.

*The Role of Nurseries in Facilitating Collection:*

Realistically, accessing *A. palmata* and *A. cervicornis* spawn from different habitats is extremely challenging. Field nurseries provide a substantial advantage by maintaining and tracking multiple, generally known genotypes in one location, facilitating collections by a single

dive team. The capacity to reliably spawn corals in land-based nurseries is also developing (Craggs et al. 2017). Thus, opportunities to collect specimens from multiple genotypes and possibly at multiple times of year in land-based spawning systems should certainly be utilized. Such collections would also have the added advantage of being linked with live-banked (holobiont) specimens of the same genotypes. However, nursery stocks may not contain the full range of genetic diversity present in a given geographic region, as the mid-water nurseries represent a distinct (selective) habitat which may not be suitable for all genotypes. It also takes substantially more investment to maintain a nursery-housed spawning stock of *A. palmata* than it does for *A. cervicornis* due to its slower growth in-nursery and larger size-at-maturity.

There is a hybrid strategy to assemble *spawning stock caches* that could facilitate sperm collection independent from a propagation nursery. Spawning stock caches could be established in several habitat types (e.g. depths or distances from shore) within a genetic region and involve transplanting (tagging and genotyping) many genotypes from dispersed sites into single patches at diveable sites to facilitate spawn collection. Ideally, the cache setup might occur a year or two prior to a major collection/archiving effort to allow establishment, grow-out, and genotyping of colonies, and would function as a functional 'restored site' in the meantime. To maximize collection of potentially rare alleles, colonies from extreme environments should be specifically targeted for inclusion in spawning stock caches.

Metadata Requirements:

The greater the resolution of metadata that accompany samples in the genetic bank, the more useful these samples are likely to be in the future. On the other hand, it may not be prudent to exclude otherwise valuable samples if only lower resolution metadata are available. Regardless, minimum metadata is required to be submitted, compiled, and made publicly available for all banked specimen submissions, requiring some dedicated curatorial effort. The proposed minimum required metadata include a unique ID number (so-called Registry ID#, potentially assigned by an outside registry authority[3] being developed in restoration field), collection date, species, location (geographic coordinates and depth of the donor colony for wild collections or basic nursery metadata for nursery collections), whether the sample consists of a single colony vs. a pooled sample, contact information for collector, permitting information, and basic fixation procedures. Key QA/QC data, particularly pre-freeze and post-thaw sperm motility and concentration are required, and therefore collection teams must include steps to quantify these parameters within the collection scheme. It is *strongly recommended* that the genotype of the individual be included. However, if genotype information is not known, an additional genetic voucher (i.e. tissue biopsy fixed in ethanol) should be deposited, possibly in a separate but metadata-linked collection, to enable later determination of genotype. Additional information on the habitat and phenotype of the donor colony are desirable but not required. These recommended parameters are delineated in Appendix 3.

[3] In development is a "Collection Registry" that will house general information on wild-collected coral samples and provide each entry with a unique accession number that will allow cross-reference between various data structures. A preliminary schema has been developed through collaborative effort across various academic, non-profit and government representatives.

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Appendix 1 Symbiont and whole-tissue banking considerations

Diverse symbionts are integral components of the coral holobiont, but present a great challenge in terms of identifying and potentially preserving the individual taxa that are crucial partners. The best characterized are the obligate primary algal endosymbionts: dinoflagellates in the family Symbiodiniaceae, which deserve consideration when developing strategies to bank coral material. Most corals acquire these symbionts from the environment during early life stages, and thus, any efforts to reconstitute coral species from cryopreserved gametes or zygotes will require provisioning of appropriate symbionts from reliable sources. Moreover, most coral-symbiont partnerships are highly specific, such that if the host species is lost, the symbiont species will be lost as well. It is therefore important to consider options for symbiont banking as well.

Live banks and nurseries clearly include entire holobionts, ensuring symbiont inclusion. However, specific symbiotic associations may differ in artificial environments. Cryobanking of coral whole-tissue samples can similarly capture intact symbiont communities, and cryopreservation of both *in vitro* (cultures) and *in hospite* Symbiodiniaceae has been undertaken successfully, (Hagedorn and Carter, 2015) but the success of this process was altered by recent bleaching and newer processes are now under development. Unfortunately, the vast majority of Symbiodiniaceae species are currently unculturable, including *Symbiodinium 'fitti,'* the dominant symbiont of *Acropora palmata* and *A. cervicornis*. Meanwhile, the changing physiological state of the algal symbionts within their increasingly stressed animal hosts (temperature and otherwise) poses additional challenges to strategic genetic banking of symbionts.

Although there is a rapidly growing understanding of the instrumental role of the microbiome in coral health and wellbeing, there is currently an inadequate basis for delineating specific microbial taxa as obligate mutualists. Thus, the best insurance strategy involves banking whole tissue samples (which would include the full suite of algal and microbial symbionts in the 'Vault' collections) as soon as reliable protocols can be determined.

Overall, effective strategies for banking symbionts and whole-tissue samples is needed, and both processes are under currently under intensive development at the Smithsonian with promising near term successes. Results of these efforts are tentatively expected by December 2019. Planning for cryo-archival capacity for endangered corals should incorporate the inclusion of these collections, along with sperm.

Appendix 2: Hypothetical Numbers Scenario:

*Acropora palmata*

| **Genetic region** | **location/subregion** | **Habitats present (sites sampled @ 10 genotypes)** | **Total (for n=10)** |
|---|---|---|---|
| Florida | Broward | none | |
| | Miami/Biscayne | Shallow fore (2 sites) | 20 |
| | | Urban seawall (1 site) | 5 |
| | Upper Keys | Nearshore/lagoonal patch (2) | 20 |
| | | Shallow crest/ fore (2) | 20 |
| | | Deep fore (2) | 20 |
| | Lower Keys | Nearshore/lagoonal patch (2) | 20 |
| | | Shallow crest/ fore (2) | 20 |
| | | Deep fore (2) | 20 |
| | | | 145 |

| | | | |
|---|---|---|---|
| Bahamas | Abaco | Lagoon/patch | 20 |
| | | Shallow fore | 20 |
| | Eleuthera | Shallow fore | 20 |
| | Nassau | Lagoonal patch | 20 |
| | | Shallow fore | 20 |
| | | urban/port | 6 |
| | Inagua | Shallow fore | 20 |
| | | | 126 |

. . .plus similar for additional genetic regions

Appendix 3: Suggested Metadata Requirements

The Working Group recommends three tiers of metadata to accompany banked specimens: required (*), strongly recommended(+), and 'valuable but not required' ( )

| | PARAMETER/INFORMATION | Notes/description |
|---|---|---|
| * | Registry/ID # | Universal colony/genotype identifier for cross reference among restoration endeavors; Hopefully there will be a common source of these designations across the species range. In the absence of a universal registry, the cryo-bank itself would assign this unique ID number to its accessioned specimens. |
| | | |
| * | Country | checklist |
| | Genetic Region | checklist |
| | population type | outplanted/restored vs. wild colony |
| * | Lat | decimal degrees |
| * | Lon | decimal degrees |
| * | location as colony OR reef? | |
| * | depth | m |
| * | habitat | checklist |
| * | Disease present in conspecifics at the site | yes/no/unknown |
| * | Disease present in other coral species at the site? | yes/no/unknown |
| | distance from shore | m |
| | | |
| * | Pooled or individual sample | For pooled samples, separate registry ID# could be obtained, and preferably linked to registry numbers for component genotypes if known assign |
| * | Date collected | |
| * | Person | |
| * | Contact info | |
| * | Institution | |
| * | Permit for collection | |
| | Permit for export/import | |
| * | Amount of sample | # of vials and volume per vial |

| | | |
|---|---|---|
| * | Fixation method | checklist |
| | Preparation methods | E.g., Cooling rate, cooling method, freezing medium |
| * | Pre-freeze sperm concentration (x$10^6$/ml) | |
| * | Pre-freeze motility | |
| * | post-thaw motility | Important QA/QC info; additional step to be planned and executed in the preservation process |
| * | post-thaw concentration | Important QA/QC info; additional step to be planned and executed in the preservation process |
| | fertilization success -Control | I.e. fresh sperm sample, against a pooled sample of eggs |
| | Fertilization success-post thaw | |
| * | Tissue type | Checklist (sperm, larvae, whole tissue) |
| * | Species | Checklist (A.palmata, A.cervicornis, A.prolifera |
| | | |
| | Colony designation | Field tag or other local/institutional identifier |
| * | Genotype method | Checklist (msat, SNP, 2bRAD);<br>NOTE, either analytical genotype info OR genetic voucher specimen is required, both is also good. |
| * | Genotype designation | cross-reference existing Acropora genotype database |
| | Genotype | genotype signature (ie. listed alleles; can include or reference existing genotype database) |
| * | Voucher biopsy available? | yes/no; voucher should be housed under same registry/ID# |
| | | |
| | Colony length | |
| | Colony width | |
| | Colony Ht | |
| | Nearest neighbor distance | |
| | % live tissue on colony | |
| | % recent mortality | |
| | disease | yes/no |
| | Disease type | checklist |
| | Bleaching | no/mild/severe |
| + | Photographs | Whole-colony and polyp/close-up scale |
| | gamete characteristics (size, bundle packaging) | |

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