NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATON

Management Plan for Caribbean Acropora Population Enhancement

National Marine Fisheries Service
Southeast Regional Office

December 2016

Abbreviations List

µmol m^{-2} s^{-1}	micromoles per square meters per second
C	Celsius
cm^2	square centimeters
ESA	Endangered Species Act
FWS	Fish and Wildlife Service
GPS	global positioning system
km	kilometers
m	meters
$mEqL^{-1}$	milliequivalent per liter
mgL^{-1}	milligrams per liter
mi	miles
ms^{-1}	meters per second
mV	millivolts
NMFS	National Marine Fisheries Service
PAR	photosynthetically active radiation
ppt	parts per thousand
PVC	polyvinyl chloride

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Introduction

Elkhorn coral (*Acropora palmata)* and staghorn coral (*Acropora cervicornis)* were once among the most abundant coral species in the Caribbean and Florida Keys, forming dense thickets and substantially contributing to accretion of reef habitat. However, since the 1980s, drastic declines throughout their range resulted in their 2006 listing as threatened under the US Endangered Species Act (ESA).

Acropora Recovery Plan

The ESA requires the development of a recovery plan for listed species to identify actions necessary for the conservation and survival of the species and criteria that would indicate the species is recovered. The Recovery Plan for Elkhorn and Staghorn Corals (*Acropora* Recovery Plan) was finalized in March 2015. Two of the actions identified in the *Acropora* Recovery Plan are active population enhancement and the development of guidelines and policies to minimize risks associated with population enhancement activities (NMFS 2015). Population enhancement can involve stabilizing unattached coral fragments or restocking using colonies derived from larval settlement or asexual fragmentation. To the maximum extent possible, population enhancement efforts must both preserve the ecological and genetic distinctiveness of listed species and minimize risks to wild populations. The two main risks identified in the recovery plan are genetic consequences and health impacts on wild populations (NMFS 2015).

There are multiple organizations involved in propagating *Acropora* spp. for population enhancement both within US jurisdiction and throughout the greater Caribbean. The intent of *Acropora* population enhancement efforts is to aid in the recovery of elkhorn and staghorn corals and to improve reef community and ecosystem function without negatively impacting native populations. Various factors, including ongoing disturbances and population decline, Allee effects, low sexual recruitment rates, and limited dispersal and genetic exchange, slow the process of unaided natural recovery. The propagation of *Acropora* corals that have survived multiple stressors under a changing climate may accelerate the otherwise uncertain recovery of these species. Thus, active population enhancement is needed to help reach the population-based recovery criteria listed in the recovery plan (NMFS 2015).

Policy Regarding Controlled Propagation of Species

When active propagation is used for recovery of ESA listed species, the National Marine Fisheries Service (NMFS) must adhere to the joint US Fish and Wildlife Service (FWS) and NMFS Policy Regarding Controlled Propagation of Species Listed under the Endangered Species Act (FWS and NMFS 2000). The policy identifies eight specific risks to evaluate for active propagation including:

1. Removal of natural parental stock that may result in an increased risk of extinction by reducing the abundance of wild individuals and reducing genetic variability within naturally occurring populations;

2. Equipment failures, human error, disease, and other potential catastrophic events that may cause the loss of some or all of the population being held or maintained in captivity or cultivation;
3. The potential for an increased level of inbreeding or other adverse genetic effects within populations that may result from the enhancement of only a portion of the gene pool;
4. Potential erosion of genetic differences between populations as a result of mixed stock transfers or supplementation;
5. Exposure to novel selection regimes in controlled environments that may diminish a listed species' natural capacity to survive and reproduce in the wild;
6. Genetic introgression (infiltration of the genes of one species into the gene pool of another through repeated backcrossing of an interspecific hybrid with one of its parents) which may diminish local adaptations of the naturally occurring population;
7. Increased predation, competition for food, space, mates, or other factors that may displace naturally occurring individuals, or interfere with foraging, migratory, reproductive, or other essential behaviors;
8. Disease transmission.

These eight risks are further discussed in the last section Risk Assessment and Management.

Purpose

The purpose of this population enhancement management plan (Management Plan) is two-fold. The first is to adhere to the Policy Regarding Controlled Propagation of Species Listed under the Endangered Species Act. The Management Plan addresses the risks identified in the policy based on the current level of knowledge and provides guidelines and best management practices to minimize risks where information is lacking. The second is to guide active propagation of elkhorn and staghorn corals to maximize recovery potential as identified by the goals, actions, and criteria identified in the *Acropora* Recovery Plan (NMFS 2015). The Management Plan pertains to any efforts to propagate *A. palmata* or *A. cervicornis* when the intention is to release propagated individuals for reintroduction or augmentation of existing populations or to establish or maintain refugia populations (populations removed from the wild).

This Management Plan will provide best practices for nursery cultivation and outplanting, guidelines for outplanting design and site selection, and a standardized approach to outplant tracking and reporting. It builds upon concepts and recommendations developed during a 2-day workshop for *Acropora* conservation and restoration hosted by the Smithsonian Institution and the National Oceanic and Atmospheric Administration in Washington DC in November 2009 (Smithsonian Institution 2009) and on best practices manuals developed by The Nature Conservancy (Johnson et al. 2011) and the Punta Cana Ecological Foundation (Bowden-Kerby 2014). Coral restoration partners, including non-governmental organizations, academia, zoos, aquaria, and federal, state, and local agencies, are requested to collectively implement this Management Plan.

Partners should coordinate with NMFS Southeast Regional Office to ensure that population enhancement guidelines are followed and that outplanting efforts are reported to document recovery across the range.

In addition, depending on the location of the activities, permits are required from federal, state, territorial, and/or local agencies for collecting fragments and gametes. Permits are also required for propagating and outplanting activities, including the use of *in-situ* structures and buoys. These permits may have guidelines, best management practices, monitoring, and reporting requirements in addition to those identified in this Management Plan.

Background

Reproduction

Acropora cervicornis and *A. palmata* can reproduce both asexually through reattachment of fragments and sexually through fertilization of gametes. Fragmentation can occur from natural events such as storms and anthropogenic causes such as anchoring, vessel groundings, and diver activities. Reattachment of fragments is likely the main mode of population maintenance and growth in many locations and can result in stands of genetically identical colonies. Colonies of both species are hermaphroditic but do not effectively self-fertilize. Though broadcast spawning occurs annually, sexual recruitment in these two species is low.

Sexual reproduction introduces new genetic individuals into the population. Dispersal of larvae affects the genetic linkage among populations and is the only way to naturally repopulate distant areas since size and weight of asexual fragments limit their dispersal ability. The *Acropora* Recovery Plan (NMFS 2015) identified depensatory population effects (negative feedbacks that occur when population levels are low) as a moderate threat to recovery. Allee effects (reduced mate availability due to distantly located, genetically distinct individuals), asynchronous spawning (Miller et al. 2016), and genotypic incompatibility (Baums et al. 2013) likely contribute to low fertilization rates and inhibit recovery. Thus, increasing density and genotypic diversity in local populations through population enhancement could aid in successful sexual reproduction necessary for natural repopulation and species recovery.

Genetics

Elkhorn and staghorn corals retain moderate to high levels of genotypic diversity (*i.e*., the ratio of genetically distinct individuals to all colonies in a population or the relative abundance of distinct genetic individuals) in many regions (Baums et al. 2010; Baums et al. 2006; Vollmer and Palumbi 2007). However, low genotypic diversity exists in some areas such as certain reefs in the Florida Keys, which are composed primarily of a single elkhorn genotype (Baums et al. 2005; Baums et al. 2006; Williams et al. 2014a). Elkhorn coral populations in the western Atlantic and Caribbean (Bahamas, Florida, Mexico, Panama, Navassa, and Mona Island) have experienced little to no genetic exchange with populations in the eastern Caribbean (St. Vincent and the Grenadines, USVI, Curacao, and Bonaire) (Baums et al. 2005).

Puerto Rico was a zone of mixing with contributions from both regions, but it had a closer genetic connection with the western Caribbean.

Regional populations of staghorn coral separated by greater than 500 kilometers (km) (310 miles [mi]) are genetically differentiated, and gene flow across the greater Caribbean is low overall (Hemond and Vollmer 2010; Vollmer and Palumbi 2007), which is consistent with studies of other coral species (Baums et al. 2005; Brazeau et al. 2005; Fukami et al. 2004). The staghorn coral population across Florida is highly genetically interconnected and, according to some studies, has no discernable genetic structure (Baums et al. 2010; Hemond and Vollmer 2010). A more recent study found genetic structure between some counties in Florida, which may be more indicative of the statistical power of the methods used versus an ecologically relevant distinction (Drury et al. 2016). Genetic variation within and among *A. cervicornis* populations in different counties in Florida is high and is similar to the genetic variation found in large outbred populations spread over large geographic ranges (Drury et al. 2016). The Florida population is distinct from other areas in the Caribbean (Honduras, Bahamas, Navassa, St. Thomas US Virgin Islands, and Puerto Rico) (Baums et al. 2010; Hemond and Vollmer 2010). The USVI and Puerto Rico populations are connected as are the populations of Navassa and the Bahamas (Baums et al. 2010). There is evidence staghorn populations in the western Caribbean (Yucatan, Belize, and Panama) are distinct from the eastern Caribbean and that Florida is genetically connected to the western Caribbean (Vollmer and Palumbi 2007), which is consistent with elkhorn coral (Baums et al. 2005).

Fine scale staghorn coral population structure can occur over spatial distances less than 100 km (in as little as 2 km) but rarely does (Vollmer and Palumbi 2007). These fine scale patterns were mostly due to introgressed genes from *A. palmata* that enter *A. cervicornis* through backcrossing with the hybrid *Acropora prolifera* and occurred in some highly localized reefs in Puerto Rico, Panama, and San Salvador (Vollmer and Palumbi 2007). No fine scale genetic structure was observed between staghorn populations in the upper and lower Florida Keys despite high frequencies of introgressed genes (Baums et al. 2010; Hemond and Vollmer 2010), though a subsequent study found fine scale genetic structure within Miami-Dade County (Drury et al. 2016). These differences are likely due to the genetic markers used; lower resolution mitochondrial DNA markers in the case of Hemond and Vollmer (2010) versus the genome-wide genotype by sequencing study employed by Drury et al. (2016).

Disease

The *Acropora* recovery plan identifies disease as one of the main causes of *Acropora* decline and an impediment to species recovery (NMFS 2015). There are several named diseases that affect elkhorn and/or staghorn coral through visual tissue loss (white band, white band type II, white pox, acroporid serratiosis, patchy necrosis, rapid tissue loss). However, most are not readily distinguishable from each other in field populations and have no known pathogen. Corallivores, such as the snail *Coralliophila abbreviata,* may act as vectors of disease (Gignoux-Wolfsohn et al. 2012; Sutherland et al. 2011; Williams and Miller 2005). Irrefutable identification of specific pathogens as the causative agent of disease has been elusive. For instance, a strain of *Serratia marcescens* has been shown to cause acroporid serratiosis (Sutherland et al. 2011), but *S. marcescens* was not detected in elkhorn corals in

the US Virgin Islands that showed lesions similar to those described for white pox (Polson et al. 2009). Hundreds of potential bacterial pathogens have been associated with white band disease on staghorn coral (Gignoux-Wolfsohn and Vollmer 2015).

Further impeding identification of causative agents of disease is the fact that corals host a variety of microbial communities. Increased disease prevalence or virulence associated with stressors (*e.g.*, high temperatures, hurricanes) suggests that suboptimal environmental conditions may increase coral susceptibility to disease by weakening their defense and allowing pathogenic microbes to invade and proliferate (Ritchie 2006). Thus, elimination of the threat of disease in population enhancement efforts is not possible given the currently limited level of knowledge regarding causative agents, pathways, and mechanisms. However, reducing other stressors (*e.g.*, land-based sources of pollution, predation, turbidity) may help minimize the effects of disease.

Propagation Methods

Because these two species can reproduce through fragmentation and gamete fertilization, both sexual and asexual propagation techniques exist. In addition, propagation can occur in *ex situ* (land-based) or *in situ* (offshore) nurseries. These techniques are described in more detail below.

For asexual propagation, a small piece of coral (generally ≤ 5 centimeters [cm]) is clipped from a wild colony (donor) and maintained in *ex situ* or *in situ* nurseries removed from common stressors such as sedimentation and predators. As the fragments grow in size, subsequent fragments can be taken from these colonies and outplanted back to the reef environment. In this way, there is a broodstock of corals that remain in nurseries and provide corals for restoration without depleting wild populations.

Corals rescued from coastal construction activities or fragments generated from physical damage events, such as storms, vessel groundings, and anchors, can also provide a source of fragments for population enhancement and restoration. Storm-generated elkhorn coral fragments exhibit higher survival when manually reattached to the substrate rather than left to reattach naturally on their own (Williams and Miller 2010). Fragments can be reattached in place or moved to nurseries for propagation and later outplanting.

Sexual propagation techniques usually involve fertilizing eggs in the laboratory from gametes collected during annual spawning events on the reef. Detailed methods for sexual propagation are outlined in the *Acropora* Coral Restoration/Conservation Workshop Final Report (Smithsonian Institution 2009). Gametes must be collected from different genotypes as staghorn and elkhorn colonies are not able to effectively self-fertilize even though they are hermaphroditic. Collecting from compatible colonies can be logistically difficult since spawning is not always synchronous and some reefs are largely composed of one clonal genotype. Eggs and sperm are released in gamete bundles, which break apart after about 30-90 minutes. Fertilization can be maximized by maintaining sperm at ideal concentrations since dilution can lead to low fertilization success. Cryopreservation of sperm provides a potential method of dealing with asynchronous spawning (Hagedorn et al. 2006) by enabling fertilization of eggs with previously frozen sperm. Fertilized eggs develop for several days until they become free-swimming planula larvae.

Larvae will then settle onto appropriate settlement substrata and can be maintained in captivity or outplanted back to the reef (Chamberland et al. 2015). Sexual propagation can be very labor intensive, but it is the only way to add genetically unique individuals to the population.

In situ nurseries are the most common way to propagate corals. Fragments of coral are maintained on benthic structures, such as concrete blocks or wire frames, or suspended in the water column on floating structures, such as PVC frames or lines. Fragments grown in nurseries can commonly achieve survivorship upwards of 90%, potentially due to the reduced stressors and optimal growing conditions.

Ex situ nurseries are commonly used for sexual propagation and larval grow-out but are also used for propagation through fragmentation. Water for *ex situ* nurseries is sourced from saltwater wells, directly from the ocean, or can be made from commercially available mixtures for artificial seawater. Similar to *in situ* nurseries, corals are maintained on benthic structures or are suspended in the water column.

There are a number of advantages and disadvantages for *in situ* and *ex situ* nurseries. *In situ* nurseries provide the advantage of employing low technology and low cost methods. They require relatively little maintenance if sited in an appropriate location and keep the corals in similar conditions to those on the reef, reducing concerns about acclimation to and selection for unnatural conditions. However, they are still subject to stressors like temperature extremes, pollution, and physical damage. Because *ex situ* land-based nurseries are easily accessible, corals can be tended at any time without the logistical constraints associated with offshore nurseries (*e.g*., weather conditions, boat and personnel availability). Water quality conditions (*e.g*., temperature, pH) can be controlled, and *ex situ* nurseries do not require proximity to coastal areas. However, they can be more costly to run, are subject to technology and equipment failure, are more space limited, and require staff knowledgeable in water quality and husbandry techniques. Both nursery types have demonstrated the advantage of being able to increase number of colonies relatively quickly and easily using asexual propagation techniques.

Population Enhancement Guidelines

Goal

The overall goal of population enhancement is to increase the abundance and genetic diversity of elkhorn and staghorn corals throughout their range, without negatively affecting wild colonies. By creating localized areas of abundant, genetically diverse, sexually reproductive colonies of elkhorn and staghorn corals throughout their range, population enhancement efforts can help overcome some of the obstacles to successful sexual reproduction constraining wild populations and can aid in natural recovery.

Objective

The objective this Management Plan is to establish a set of operating guidelines on which the coral restoration community can rely to systematically and reliably achieve the goal of population enhancement. The guidelines in this Management Plan will reduce risks and maximize the chances of successful population enhancement by following the actions and criteria identified in the *Acropora* Recovery Plan. Population enhancement of elkhorn and staghorn coral will use a multi-faceted approach that builds upon the existing efforts currently being conducted by various non-government, academic, and government organizations. Since multiple entities are involved, there is a need for coordination to ensure best practices are followed and for quality control, data management, and tracking. Both sexual and asexual propagation methods, as well as *in situ* and *ex situ* nurseries, should be used in a number of locations throughout the species' range. Using a variety of methods and techniques in multiple locations will maximize production of genetically distinct individuals available for population enhancement efforts and reduce the risk of catastrophic events (*e.g*., storms, temperature extremes, equipment failure) affecting a large portion of the available stock.

Health Considerations

Because coral disease etiology is largely unknown at the current time, the best management practices section identifies several strategies for reducing the risk of disease introduction or transfer. As new information becomes available, these practices can be updated as needed and will appear in Appendix B. Disease management guidelines focus on reducing the risk of disease introduction when placing corals into a nursery environment, reducing the spread of disease while in culture, and minimizing the risk of disease when outplanting back to the reef. A visual health assessment process has been developed for the Special Activity License issued by the Florida Fish and Wildlife Conservation Commission for collecting and outplanting *Acropora* corals, and the guidelines described here build upon their efforts. It is unlikely that the risk of disease will be eliminated during culture and other population enhancement activities. Disease is believed to be more prevalent in stressed corals, and there is some stress associated with handling and moving corals. Also, disease is endemic in the wild population, and global climate stress continues to impact wild, cultured, and outplanted corals alike. However, implementation of these management strategies will assist in reducing the incidence and the possibility of spread of disease.

Genetic Considerations

With any population enhancement program, one of the factors to consider is how selective propagation will affect the genetic structure of restored populations. Asexual propagation methods that can produce a large number of genetically identical clones are of particular concern because of the possibility of swamping the population with a small number of genotypes. For this reason, each nursery should aim to culture as many genotypes as possible. Nursery operators should aim to collect fragments from as many physically separated reef areas as possible to increase the chances of obtaining unique genotypes for propagation efforts. The *Acropora* Recovery Plan (NMFS 2015) advocates maintaining or achieving a genotypic diversity (number of unique genotypes per number of colonies sampled) close to 0.5 in the

wild throughout the range of these two species. This would indicate a balance between sexual and asexual recruitment (a genotypic diversity equal to one would indicate purely sexual recruitment while a genotypic diversity approaching zero would indicate predominantly asexual recruitment). Population enhancement efforts should aim to outplant as many genotypes as feasible. Population enhancement efforts that use fragmentation will push genotypic diversity at individual sites towards a value less than 0.5. However, outplanting a number of different genotypes in close proximity will increase the chances of successful sexual reproduction and larval settlement that will help achieve the range-wide genotypic diversity closer to 0.5.

Another prudent practice is to house individual genotypes in several physically separated locations within a state, territory, or country, in both *ex situ* and *in situ* nurseries, if possible. This practice will reduce the risk of losing entire genotypes due to catastrophic events. As coordination among nursery operators and managers increases, it might be possible to move coral fragments across political boundaries and house some genotypes in other states, territories, or countries. However, the initial focus should be to replicate across multiple nurseries within a state, territory, or country.

Genetic diversity of the microbial and algal communities (zooxanthellae) that reside within the coral host is another consideration. Caribbean coral microbial communities show strong phylogenetic similarities within coral genera and differences across genera and sites (Chu and Vollmer 2016). *Acropora palmata* and *A. cervicornis* associate predominantly with one symbiont species (*Symbiodinium* "*fitti*") (Baums et al. 2014). Most colonies of a particular *A. palmata* genotype are dominated by one symbiont genotype (or strain) that may persist in the host for decades or more (Baums et al. 2014). Gene flow among symbiont populations is an order of magnitude lower than gene flow among *A. palmata* (Baums et al. 2014). Further, there is a growing body of evidence that host genotype-by-symbiont genotype interactions may provide significant physiological variation, influencing the adaptive potential of symbiotic reef corals to severe selection (Parkinson et al. 2015). Microbial communities are not passed from parent to offspring in staghorn and elkhorn corals (Sharp et al. 2010). Therefore, sexually propagated corals must uptake them from the surrounding environment. Microbial communities are likely acquired after larval settlement (Sharp et al. 2010), but zooxanthellae may be acquired during the larval stage. Therefore, the best practice for maintaining diversity of coral-associated organisms is to culture larvae in water derived from the region of gamete collection or to add fragments of substrate conditioned for a few weeks in water from the region of gamete collection to the larval culture system. This practice will increase the chances that native zooxanthellae and microbial communities are available for uptake. However, more sophisticated methods of algal and microbial community culture and inoculation should be developed.

One of the actions of the *Acropora* Recovery Plan is to research and develop mechanisms to enhance adaptation/acclimation of elkhorn and staghorn corals to increases in climate stress. One of the potential ways to accomplish this action is to apply selection in culture and outplanting efforts to focus on genotypes that appear to be more resistant to the effects of temperature stress, disease, and ocean acidification. Alternately, targeted translocation of genotypes from thermally variable sites is another potential way to enhance adaptation/acclimation to increases in climate stress since there is evidence

that corals in more thermally variable environments may be more tolerant to changes in temperature. Testing genotypes for resistance or resilience to climate stress and disease should be undertaken, but maintenance of genetic diversity should be a primary concern in both culture and outplanting. Trade-offs in performance may occur, and genotypes that do not appear to perform as well should not be completely abandoned (reviewed in Baums 2008).

Another possibility for enhancing adaptation/acclimation of elkhorn and staghorn corals to increases in climate stress may be to breed resistance characteristics into them through outcrossing with more resistant genotypes. *Acropora palmata* and *A. cervicornis* naturally interbreed to produce the hybrid *A. prolifera*. *Acropora prolifera* is not present in the geologic record but is presently abundant in some locations. There is some discussion in the restoration and research community of possibly backcrossing the hybrid with *A. cervicornis* and *A. palmata* to breed more robustness into the species, though evidence for hybrid vigor is rudimentary. Because climate stress is expected to continue to increase, research and experimentation on hybrid vigor and identifying the potential genetic basis is warranted. However, for population enhancement purposes, the preferred path is to outcross more resistant/resilient genotypes of the same species to preserve genetic distinctiveness and limit introgression of genes of one species into the other.

Culture Considerations

There are several methods currently employed for *in situ* nursery culture of corals. Fixed structures such as concrete blocks, engineered bases or stands, and polyvinyl chloride (PVC) pipe or metal racks have been used successfully. However, floating structures, such as line nurseries and suspended PVC trees, often have higher growth rates and less predation and sedimentation stress. Any nursery structure that uses line has the potential risk of entanglement with marine life, which may have consequences if it is a protected species like threatened and endangered sea turtles. See the best management practices for guidelines for reducing the risk of entanglement.

Several practices are common to both fixed and suspended structures. Nurseries should be located in sand away from coral reefs. Regular maintenance like algae removal will likely be necessary although in some instances herbivorous fish (parrotfish and surgeonfish) will graze the structures to help control fouling. If excessive fouling occurs, an alternate nursery site may need to be considered if herbivores are unable to keep algae in check. Experience has shown it is better to keep corals in nurseries trimmed to smaller sizes (approximately less than 25 cm (about 9.84 inches [in]) in diameter) since larger colonies are more likely to crowd other colonies, causing competition and stress or breakage. All colonies in nurseries should be tracked for genetic relationships (*i.e*., track which fragments came from which parent colony).

For *ex situ* nurseries, water quality, water flow, temperature, and light levels are all important factors influencing coral condition and growth. Fewer entities have worked on culturing *Acropora* species in *ex situ* land-based systems for population enhancement activities, but water quality conditions needed to keep corals in captivity are generally known (Table 1). Water flow rates should be alternating and

turbulent if possible, and a good rule of thumb is to have at least 10 times the tank volume per hour in circulation (O'Neil 2015). Water flow of 0.2 – 0.7 meter per second (ms^{-1}) was good for growth of *A. cervicornis* in one study using an *ex situ* nursery (O'Neil 2015). Lower water velocity may be suitable for keeping corals alive but could have trade-offs associated with growth, branching, and skeletal density. Light levels should be comparable to those found in natural habitat. *Acropora cervicornis* is most common in 5-20 meter (m) depth, and *A. palmata* is most common in 1-5 m depth. Light level (photosynthetically active radiation [PAR]) good for growth of *A. cervicornis* was between 450 and 500 micromoles per square meter per second ($\mu mol\ m^{-2}\ s^{-1}$) as measured at the coral branches in one study of growth in a land-based nursery (O'Neil 2015). Other light levels may be acceptable, but there may be trade-offs associated with coral growth, nuisance algal growth, and amount of maintenance needed to keep nuisance algae under control.

The ability to maintain stable water quality conditions is also important, as corals are intolerant of rapid fluctuations. A good rule of thumb is that if any water quality parameters are out of range for an extended period of time (*i.e.*, days), they should be brought back into range slowly and with only one parameter change at a time unless corals are in imminent danger (O'Neil 2015). If a recirculating system is used, essential components of an effective life support system include a biological filter (such as live rock) for removal of nitrogenous compounds, protein skimmer, calcium reactor (or other source of calcium and alkalinity), temperature control, a dechlorinated and purified water source (*e.g.*, reverse osmosis), and a reliable back-up power generation system. Depending on the water source, some of these components may not be needed in a flow-through system. Herbivores such as snails should be considered as a way to keep nuisance algae under control and lessen the need for manual algae removal. Herbivores and live rock should be sourced from the same area in which the corals originated.

Table 1. Water quality parameters[1] commonly measured in coral aquaculture and recommended ranges for *A. cervicornis* (from O'Neil 2015).

Parameter	General Recommended Range	*Acropora cervicornis* Ideal Range	Notes on Life Support Design and Maintenance
Total Ammonia (NH_3)	0 - 0.1 mg L^{-1}	<0.03 mg L^{-1}; Reduced extension seen at 0.28 mg L^{-1}	Well established biological filtration, clean seawater and RO water source should minimize problems
Nitrite (NO_2)	0 - 0.1 mg L^{-1}	<0.03 mg L^{-1}	Well established biological filtration, clean seawater and RO water source should minimize problems
Nitrate (NO_3)	0 - 1.0 mg L^{-1}	<1.0 mg L^{-1}; caution to avoid complete removal or fast reduction	Anaerobic areas such as deep sand or porous rock must be provided or export through algae growth; use of denitrification filters possible but not explored
Phosphate (PO_4)	0.00-0.05 mg L^{-1}; some <0.03 mg L^{-1}	0.02-0.05 mg L^{-1}; caution to avoid complete removal or fast reduction	Dilute lanthanum chloride additions used with no ill effect; caution on un-secured iron oxide hydroxide media potentially causing irritation
Total Alkalinity	3.0 - 4.0 mEq L^{-1} 150-200 mg L^{-1} as $CaCO_3$	Same, maintain a high aragonite saturation state without precipitation	Both calcium and total alkalinity should be maintained through the use of a calcium reactor; additions of kalkwasser, calcium chloride, sodium bicarbonate and/or other buffering agents can also be used but may not be suitable for maintenance in heavily stocked, fast-growing systems
Calcium	350-450 mg L^{-1}	Same, maintain a high aragonite saturation state without precipitation	
Temperature	25.0 - 28.0˚C, minimum 18˚C	Minimum of 26˚C to avoid seasonal decrease in extension rate, maximum of 29˚C to avoid summer temperature stress	Heating and cooling system must be adequately sized to maintain temperature within ±1˚ of setpoint even in extreme weather conditions; temperature manipulations may be necessary to induce gamete production or control disease outbreaks
pH	8.0 - 8.4	>8.20 for best growth	Expected to stay within range if alkalinity values are in range and gas exchange is sufficient
ORP/Ozone	300 - 350 mV	<325 mV; very low dosage of ozone or no ozone use	Negative effects on *A. cervicornis* such as retracted polyps and expulsion of mesenterial filaments were seen at high doses; possibly related to ozone dosage into seawater with ammonium present; higher dosage has potential benefit in disease control if applied only with low N
Salinity	33-36 ppt	unknown, large fluctuations to 30 ppt or less caused stress in new nursery	Stable salinity of seawater source; availability of dry salt and clean RO water for adjustments at all times; benefit of being under shelter to avoid rainfall fluctuations; possible benefit of having a peaked roof if only using shade cloth as cover

1. Units of measurement include milligrams per liter (mgL^{-1}); milliequivalent per liter ($mEqL^{-1}$); Celsius (C); millivolts (mV); and parts per thousand (ppt).

Outplant Considerations

Site Selection

When selecting sites for outplanting, there are several factors to consider, two of which are depth and reef zone. *Acropora palmata* most commonly inhabits shallow depths of 1-5 m though it is rarely found to 30 m. In general, outplant locations for *A. palmata* should be less than 12 m in depth and ideally be in depths of 1-5 m where wild colonies most commonly occur. Optimally, the outplant site should be located in similar water depth and have similar physical conditions (e.g., light availability, water quality, water circulation) to those at the collection site. Corals should be acclimated to changes in light level if they are going to be outplanted to areas much different in depth from those in which they were collected and cultivated. The *Acropora* Recovery Plan identifies that approximately 10% of the consolidated reef habitat in 1-5 m water depth within the forereef zone should be occupied by thickets of *A. palmata* as an indicator of recovery of the species, so outplanting efforts should target this depth and reef zone.

Acropora cervicornis most commonly inhabits depths of 5-20 m though it is rarely found to 60 m. Outplanting of *A. cervicornis* should generally occur in depths less than 30 m and ideally be in depths of 5-20 m where wild colonies most commonly occur. Optimally, the outplant site should be located in similar water depth and have similar physical conditions (e.g., light availability, water quality, water circulation) to those at the collection site. A modelling study in the Florida Keys found that *A. cervicornis* is more likely to persist in areas with an average of 60% of surface PAR reaching the benthos (Ames 2016). Corals should be acclimated to changes in light level if they are going to be outplanted to areas much different in depth from those in which they were cultivated. The *Acropora* Recovery Plan identifies that approximately 5% of the consolidated reef habitat in 5-20 m water depth within the forereef zone should be occupied by thickets of *A. cervicornis* as an indicator of recovery of the species. Outplanting efforts should target this depth and reef zone. In Florida however, where most extant populations are in nearshore shallow habitats and patch reefs (Miller et al. 2008), nearshore and patch reef areas should also be targeted for outplanting efforts in addition to the forereef zone.

Other aspects to consider when choosing outplant sites include historical presence of *Acropora* and presence of stressors. Reefs where the species historically occurred are good candidates for outplant sites since conditions conducive to growth and survival existed at these sites in the recent (~50 years) past. The presence of live *Acropora* colonies, dead standing skeletons, or accumulation of obvious *Acropora* rubble can be good indicators of past existence at a reef site. However, if stressors such as poor water quality (poor water clarity or high sediment load) currently occur, it would be practical to choose sites with better environmental conditions. Sites with the presence or high abundance of coral predators such as three spot or yellowtail damselfish (*Stegastes planifrons*, *Microspathodon chrysurus*), fireworms (*Hermodice carunculata*), and snails (*Coralliophila abbreviata*) should be avoided. There should be open space to outplant that is free of organisms that may stress or overgrow outplants if they contact them (*e.g.*, encrusting gorgonians, *Palythoa caribaeorum*). Areas with excessive sand, fine-grained sand, or turf algae that can bind sediment should be avoided.

The Nature Conservancy has developed evaluation criteria to rate resilience factors at potential outplant sites in the US Virgin Islands (Table 2). A modified Atlantic and Gulf Rapid Reef Assessment (www.agrra.org/training-tools/) survey is used to collect data, but some attributes (*e.g*., water quality, water flow) are qualitatively evaluated. Positive attributes are given a higher numerical score and include good water quality, living acroporids, presence of coral recruits, good water flow, presence of crustose coralline algae, diverse coral species, presence of *Diadema antillarum* urchins, presence of herbivorous fish, and available space. Negative attributes are given a lower score and include excessive macroalgae, coral disease, coral bleaching or paling, abundant *H. carunculata* fire worms, presence of three spot or yellowtail damselfish (*S. planifrons* and *M. chrysurus*), and presence of *C. abbreviata* snails. Attributes that are thought to be more important by those conducting the site surveys can be given more weight. All factors for a site are added together, and sites that rank highest are targeted for outplanting efforts. The Nature Conservancy has found better outplant survival at sites with higher resilience scores.

Table 2. The Nature Conservancy evaluation criteria for selecting outplanting sites.

Criteria	**Measure**	**Score**		
		3	**2**	**1**
Water Quality	Local area knowledge	no issues	moderate issues; typically after rain events	known issues and point sources of discharge
Flow	Local area knowledge	constant flow	moderate flow	lagoonal; sometimes still
Acroporids	Measured abundance	>50 colonies	25-50 colonies	<25 colonies
Coral Assemblage	Measured % cover and diversity	>20% coverage and >50% coral genera	>20% coverage or >50% coral genera	<20% coverage and <50% coral genera
Diadema	Measured abundance	>50	25-50	<25
Damselfish	Measured % predation mark per colony	<5%	5-15%	>15%
Macroalgae	Measured % coverage	1-5%	6-10%	>10%
Corallivores	Measured abundance	0	1-15	>15
Health	Measured % bleaching and paling	0%	1-20%	>20%

A good practice to follow when outplanting to a new site is to do a small-scale test outplanting and see how the corals fare. If high mortality or breakage occurs, it might be better to choose a different outplant site. If the corals survive well after about six months, the site is a good candidate to receive more outplanted corals.

Predator control methods may prove effective at some sites. A capped PVC pipe with a hole drilled in one end and baited with squid has been an effective means of trapping fireworms in the Dominican Republic (Bowden-Kerby 2014). The traps can be placed at the beginning of a dive and removed at the end. Any fireworms trapped inside can be discarded on land. Corallivorous snail (*C. abbreviata*) removal from an *A. palmata* population in the Florida Keys resulted in less tissue loss compared to controls where the snail was left in place (Miller 2001). However, removal of *C. abbreviata* will have to be repeated periodically to be most effective since snails will return, particularly at sites where they were initially found to be abundant on existing *Acropora* spp. colonies (Williams et al. 2014b). Though these snails occur on other coral species, removals can be more efficiently focused on existing *Acropora* spp. colonies since there is no additional benefit of removing them from all host coral species (Williams et al. 2014b).

Outplant Design

Some successful techniques for outplanting include attaching colonies to the substrate with epoxy or cement, attaching a base on which the colony has grown to the substrate with cement or epoxy, and cable tying colonies to nails hammered into the substrate. For all methods, removing turf and macroalgae from the substrate using a brush facilitates attachment. Other methods that do not attach fragments directly to the substrate have also been used including wedging small fragments into cracks and crevices in the reef and pegging a rope with entwined fragments to the reef (Bowden-Kerby 2014). Methods that do not directly attach fragments to the substrate may have a higher failure rate. However, because they are often faster, the decreased success rate may be outweighed by the ability to put more fragments on the reef in a shorter amount of time, particularly at times when calm seas are expected in the immediate future (5-7 days) and growth rates are relatively high (summer months). The best method to use at a particular site may be determined with a test outplanting of a small number of fragments.

Size of outplanted colonies is another factor to take into consideration. In Florida, most projects outplant *A. cervicornis* colonies between 20 and 50 cm total linear extent (approximately 5-10 cm diameter) or between 50 and 100 cm total linear extent (approximately 10-15 cm diameter). Larger outplanted colonies are usually expected to perform better than smaller colonies, but they require a larger investment of time and money to grow in nurseries. In Florida, there was no significant difference in performance (growth and mortality) of *A. palmata* colonies (one genotype) in small (13.4-75.5 square centimeters [cm^2]) and large (76.0 -210.3 cm^2) size classes over short time scales (6 months), though monitoring was performed during a bleaching event that may have overwhelmed expected size differences (Pausch et al. 2015). Size performance assumptions should be tested over a wider range of

sizes and over longer periods to determine if outplanting larger colonies is worth the added effort and expense.

A limited number of studies have looked at population dynamics and run projection models to examine the effects of density and/or outplant size on population growth. For the Florida Keys, Vardi et al. (2012) projected that outplanting a higher density (1.3 colonies per square meter [m^2] per year) of smaller *A. palmata* colonies (6.5 cm diameter, <100 cm^2) for a five year period did not lead to a higher percent cover than outplanting fewer larger colonies (19 cm diameter, 100-900 cm^2) at a lower density (0.4 colonies per m^2 per year). This is because smaller colonies did not have a strong influence over population growth. Projections produced by Mercado-Molina et al. (2015) from studies in Puerto Rico indicate that outplanting *A. cervicornis* colonies 250 cm in total linear extent (approximately 35 cm in diameter) would result in a higher population size than outplanting smaller fragments. However, due to the time required to grow colonies to this size, they recommended outplanting colonies about 100 cm total linear extent (approximately 15 cm in diameter) because they can be produced faster and in larger quantities in nurseries.

Various outplant designs of colony arrangement, spacing, and density have been tried, and there does not appear to be a "best" way to outplant for all situations. A common method in Florida is to create a small cluster of several colonies of the same genotype spaced about 10-20 cm apart within a 0.3 to 1.0 m^2 plot. Clusters of a different genotype are then planted about 1 to 2 m away to promote successful cross-fertilization at future reproduction events. A design common for research is to have gridded plots of mixed genotypes with equal spacing between colonies. Other designs include planting along a transect, haphazardly placing in cracks and crevices, and overlapping colonies to form a thicket. Projects in Florida undertaken by the Coral Restoration Foundation and the Florida Fish and Wildlife Conservation Commission that have tried overlapping or stacking colonies to create a thicket have generally deemed this method unsuccessful due to high mortality.

Regardless of which colony size and arrangement is chosen, the goal should be to outplant several genotypes at a site and to arrange the colonies so that they grow to meet the abundance criterion in the *Acropora* Recovery Plan.

The abundance criterion for A. palmata is that thickets should occupy approximately 10% of the forereef zone between 1 and 5 m depth. Thickets of A. palmata are defined as either colonies at least 1 m diameter in size at a density of one colony per 4 m^2 or live percent cover of approximately 60%.

The abundance criterion for A. cervicornis is that thickets should occupy approximately 5% of the forereef zone between 5 and 20 m depth. Thickets of A. cervicornis are defined as either colonies at least 0.5 m diameter in size at a density of one colony per m^2 or live percent cover of approximately 25%.

Note that the amount of habitat identified in the abundance criterion in the recovery plan is for the entire range of the species. Therefore, the amount of habitat where outplanting is undertaken on an individual reef could be larger to help achieve the habitat occupation portion of the abundance

criterion. For instance, in a situation with 10 reefs of roughly the same size in the forereef zone between 1 and 5 m depth within a region, outplanting *A. palmata* on 10% of each reef or outplanting *A. palmata* on 20% of five reefs would both achieve the habitat occupation portion of the abundance criterion for that region. In addition, the abundance criterion addresses presence of thickets due to their ecological function, but the *Acropora* Recovery Plan assumes that lower density stands would occupy additional habitat. The use of existing habitat maps that provide information on the location and size of habitat types can be useful in making area calculations for designing outplanting to help achieve the abundance recovery criterion.

Best Management Practices

The following set of best management practices have been developed to: 1) reduce the impact of collection and propagation of corals in nurseries on wild populations of corals and other marine life, 2) reduce the chances of introduction and spread of disease, 3) increase the chances of successfully culturing and outplanting colonies, 4) ensure genetic diversity and species integrity is maintained both in wild and outplanted populations, and 5) aid in record keeping and tracking of collected, propagated, and outplanted corals. It builds on other efforts including the Florida Fish and Wildlife Conservation Commission permit guidelines and best practices manuals developed by The Nature Conservancy (Johnson et al. 2011) and the Punta Cana Ecological Foundation (Bowden-Kerby 2014). These best management practices will be updated as needed to reflect the current state of knowledge as this management plan is implemented. Updates to the best management practices will be presented in Appendix B. The most recent version of this management plan will be posted on the NMFS Southeast Regional website (http://sero.nmfs.noaa.gov/protected_resources/coral/). Additional management practices may be required depending on location and permitting agency (or agencies), and permit(s) may be required for the activities listed below.

Ex Situ Nursery Design

- Separate tanks with their own water treatment system should be incorporated for quarantine purposes.

- If a recirculating system is used, essential components of an effective life support system include a biological filter (such as live rock) for removal of nitrogenous compounds, a protein skimmer, a calcium reactor (or other source of calcium and alkalinity), temperature control, a dechlorinated and purified water source (e.g., reverse osmosis), and a reliable back-up emergency power generation system. Depending on the water source, some of these components may not be needed in a flow-through system.

- Water quality testing should occur at least weekly, but daily if possible. Automated systems that continuously monitor water quality are very useful but can be expensive. See Table 1 for suitable water quality values.

- Water flow should be alternating and turbulent if possible. Alternating and turbulent water flow can be achieved by using multiple powerheads that rotate direction and that are positioned on opposite sides of the tank.

- At least 10 times the tank volume per hour should be in circulation; lower flow may result in slower growth and poorer water quality.

- Light levels should be comparable to those found in natural habitat. Light level with a PAR between 450 and 500 µmol m^{-2} sec^{-1} is good for *A. cervicornis* growth, though light levels outside this range may be sufficient.

- Herbivores such as snails should be used as a way to keep nuisance algae under control and lessen the need for manual algae removal. Herbivores should be Caribbean species and not indigenous to the Indo-Pacific.

In Situ Nursery Design

Floating Structures

- Coral nursery structures that use lines as part of the support system or for attaching corals must be constructed in a manner to reduce the chances of entanglement with sea turtles and marine mammals.

- Line nurseries must have, at a minimum, either horizontal or vertical components that are rigid (*e.g.*, PVC pipe) in order to prevent the structures from collapsing and potentially causing entanglement of wildlife.

- Horizontal lines must be kept taut and supported by a rigid frame structure (PVC or similar) in order to avoid slack in the horizontal lines.

- Horizontal lines used to hang corals must be at least 20 cm apart to prevent entanglement of marine life.

- Vertical lines for anchoring structures to the seafloor must have sufficient tension provided by buoys to avoid slack in these lines.

- Buoys tied to the rigid component must have less than 50 cm of line exposed between the buoy and the structure.

- Line or cable that vertically attaches corals to the nursery structures must be no longer than 20 cm.

Benthic Structures

- Benthic structures must have sufficient stability to avoid being moved by storm waves. This can be achieved with the use of weight and/or penetrating anchoring systems such as "Duckbill" or "Helix" anchors or rebar driven to sufficient depth to prevent movement or lifting.

- Benthic structures must be placed at a sufficient distance from natural reef or hardbottom to avoid the potential of impacting benthic habitat from movement caused by storm waves. A suggested minimum distance is 10 m (33 ft), but a greater distance may be warranted depending on the size and type of structures.

- Benthic structures must not be placed on seagrass beds.

Collection of Fragments

- Fragments should be taken from colonies without visible signs of recent tissue loss (indicated by skeleton with visible calyx structure) that could indicate predation or a disease condition. However, with the widespread occurrence of disease and predation, it may be difficult to find unaffected colonies. If this is the case, fragments should be taken from branches that have no signs of recent tissue loss.

- For controlled asexual propagation efforts, fragments should be taken from multiple reef areas to maximize potential for sampling genetically distinct individuals. If possible, each nursery should target a minimum of 15 genotypes per species to culture but should aim to collect fragments from as many physically separated reef areas as possible to increase the chances of obtaining unique genotypes for propagation efforts. Information on site location (global positioning system [GPS] coordinates) and depth should be recorded for all collected corals.

- No more than 20% of the live tissue of a colony should be removed when taking fragments for controlled propagation. However, if fragments are generated through chance events (storms, physical impact, etc.), the whole portion of the colony that is unattached can be reattached in place or fragmented and placed in a nursery environment.

- During transportation to nurseries, fragments should be kept in water. Avoid excessive heating and exposure to sunlight to reduce transportation stress.

- Genetic analysis to identify genotypes of *Acropora* and the zooxanthellae *Symbiodinium* is strongly encouraged if funds are available. A small sample (2-3 polyps) should be collected from donor colonies, stored in 95% non-denatured ethanol, and kept frozen (if possible). Do not use rubbing alcohol available in pharmacies. If non-denatured ethanol is not available, high-percentage consumable alcohol (e.g., 80 proof [40%] rum or vodka) preserves samples for a short time until 95% ethanol can be secured. Completely immerse the sample, along with a piece of paper with the date, sample ID, and location written in pencil, in a sealed vial (ethanol will make most labels written in pen illegible over time). Tools must be sterilized (e.g., rinsed in

a 5% bleach solution and then rinsed in fresh water) between collections to avoid sample contamination.

- Fragments must be given a unique identification based on the donor colony from which they originated, and GPS coordinates and depth must be recorded for each donor colony.

- For controlled asexual fragmentation of wild donor colonies, fragment collection during the cooler months of October through May is preferred to reduce the risk of temperature stress. Collection should not occur during extreme cold events.

- Fragments of opportunity that originate from chance events like storms or vessel groundings may be reattached at their reef of origin but may not be transplanted to other reefs without first being placed in quarantine for a minimum of one month (described below in 'culture' section). If recent mortality could be from disease rather than from lying on the seafloor, the fragment should not be relocated to another reef or brought into a nursery.

- When practical, fragments originating from the same donor colony should be placed in multiple locations (*e.g.*, both *ex situ* and *in situ* nurseries, several *in situ* nurseries, several *ex situ* nurseries).

Collection and Crossing of Gametes

- GPS coordinates and depth must be recorded for each site from which gametes are collected.

- Because of the significant clonal structure of adult *Acropora* populations, gametes should be collected from colonies that are at least 15 m apart and possibly from neighboring reefs. Preferably, colonies should be genotyped before crosses are attempted.

- Because the goal is to produce as much genetic diversity as possible, it is best to have approximately equal contribution of gametes from each genotype. Batch cultures with contributions of more than two parental genotypes usually perform better than crosses between just two parental genotypes. It is not known if there is an optimal number of donor genotypes to ensure highest survival of cultures.

- Larval cultures are sensitive to temperature and salinity fluctuations as well as chemical pollutants such as plasticizers from new plastic culture dishes, sunscreen, and mosquito repellent; thus, these water quality parameters need to be controlled during fertilization and larval culture. Use glass or polystyrene whenever possible.

- Crossing of either *A. palmata* or *A. cervicornis* gametes with gametes from the hybrid *A. prolifera* should only be used for experimental purposes and should not be used for population enhancement efforts without a research component. The same applies for hybrid crosses between *A. palmata* and *A. cervicornis*.

- Because there is limited genetic transfer between regions separated by greater than 500 km, gametes from colonies separated by more than 500 km should only be crossed for experimental purposes and should not be used for population enhancement efforts without a research component.

- Once settled, corals may be maintained in nurseries (*in situ* and/or *ex situ*) or outplanted to the reef (see 'outplanting' section below).

Culture

In Situ

- Fragments should not be moved to an *in situ* nursery located more than 500 km from their point of origin in the wild.

- Because they have been shown to act as vectors, coral predators (*e.g*., *H. carunculata*, *C. abbreviata*, *S. planifrons*, *M. chrysurus*) that appear in nurseries should be removed to minimize the risk of disease transfer.

- When new fragments are placed in nurseries with existing corals, new fragments should be quarantined for a minimum of one month by placing them on separate nursery structures downstream of existing corals in the nursery.

- After one month in quarantine, corals that exhibit a visual absence of abnormal conditions (*e.g*., active tissue loss, discoloration, paling/bleaching, parasites) may be integrated into the nursery population. Corals that exhibit abnormal conditions should remain in quarantine until abnormal conditions subside and are absent for a minimum of one month.

- Any corals that have been deemed surplus to the program or recovery needs (*e.g*., to prevent genetic or ecological swamping) may be maintained in nurseries indefinitely, used for research or educational purposes, added to genetic banks, or destroyed after all other options are exhausted.

- Colonies showing active signs of disease should be treated if possible. Treatment can consist of removal of infected portions of the colony or covering infected portions with epoxy. Any diseased tissue must be destroyed or preserved for research. Living portions of diseased colonies should be quarantined as described above. If they remain disease-free for one month, they can be introduced back into the nursery. If they show signs of active disease while in quarantine, they should be re-treated as above if there is enough healthy-appearing tissue or destroyed if most of the tissue is diseased.

- When handling diseased corals, disposable gloves must be worn, removed immediately after use, and thrown away before contacting any other colonies or surfaces that might contact

healthy corals. Any surfaces, tools, or equipment that contact diseased colonies must be rinsed in a 5% bleach solution and then rinsed in fresh water before using on any other colony.

Ex Situ

- Corals originating from different states/territories or countries must be kept in tanks with separate water treatment and holding systems to prevent mixing of water or introduction of foreign species from separate geographical regions of origin.

- Before placing corals into an *ex situ* nursery, the holding tanks must be drained and equipment cleaned (scrubbed and rinsed in freshwater, and filters changed) if organisms originating from a different state/territory or country were previously held in the system.

- Non-native (*e.g*. Pacific, Red Sea) organisms may not be kept with cultured corals.

- When culturing sexually derived colonies through gamete crosses, larvae should be cultured in water derived from the region of gamete collection, or substrate (*e.g*., rubble, settlement surfaces) conditioned for a few weeks in water from the region of gamete collection should be added to the culture tanks during larval development and early settlement. This will provide access to native microbial and zooxanthellae populations that are acquired in early life stages.

- If fresh food is used to feed any organisms, it must be caught from the same state/territory or country of origin as the corals. Frozen or commercially produced dried food may be used regardless of its place of origin.

- Before new fragments are placed in nurseries with existing corals, new fragments must be quarantined for a minimum of one month by placing them in tanks with separate water treatment and holding systems to prevent mixing of water.

- After one month in quarantine, corals that exhibit a visual absence of abnormal conditions (*e.g*., active tissue loss, discoloration, paling/bleaching, parasites) may be mixed with other corals following the regional separation protocols above. Corals that exhibit abnormal conditions should remain in quarantine until abnormal conditions subside and are absent for a minimum of one month.

- Any corals that have been deemed surplus to the program or recovery needs (*e.g*., to prevent genetic or ecological swamping) may be maintained in nurseries indefinitely, used for research or educational purposes, added to genetic banks, or destroyed after all other options are exhausted.

- When working in tanks with separate water treatment systems, equipment (*e.g.*, brushes, siphons, cameras, buckets, pumps) must be soaked for 2 minutes in freshwater (soaking can be skipped if equipment is too large), thoroughly rinsed in fresh water, and dried before being transferred between tanks.

- When working in *ex situ* nurseries with separate water treatment systems, skin exposed to water while working in the tanks must be washed in soapy water to prevent unintended transfer of organisms between tanks containing corals originating from different regions.

- After handling corals that show evidence of abnormal conditions such as recent tissue loss, abnormal color, paling, etc., hands must be thoroughly washed in soapy water before touching apparently healthy corals. Alternately, gloves should be worn when handling corals displaying abnormal conditions and thrown away before contacting any other colonies or surfaces that might contact healthy corals.

- Any tools used in tanks containing corals showing abnormal conditions must be rinsed in a 5% bleach solution and then rinsed in fresh water.

- Colonies showing active signs of disease should be treated. Treatment can consist of removal of infected portions of the colony, covering infected portions with epoxy, or treatment with medicines. If using medicines, treatment must occur in an isolated treatment tank. Any diseased tissue must be destroyed or preserved for research. Living portions of diseased colonies should be quarantined by placing them in tanks with separate water treatment and holding systems. If they remain disease-free for one month, they can be introduced back into the nursery. If they show signs of active disease while in quarantine, they should be re-treated as above if there is enough healthy-appearing tissue. If most of the tissue is diseased, they should be preserved for research or destroyed.

Outplanting

- Corals must remain visually free of abnormal conditions (*e.g.*, recent tissue loss, discoloration, paling/bleaching, parasites) for a minimum of one month prior to outplanting.

- If a disease-like event (occurrence of lesions/tissue loss) affecting at least 30% of the corals in a nursery has occurred within the last month, no corals, even if they show no abnormal visual signs, can be outplanted for at least a month after the disease-like event has subsided (conditions have returned to a historical baseline for the nursery).

- Outplanting during stress events, such as high or low temperature anomalies, disease outbreaks, and algal blooms, should be avoided to reduce stress and potential susceptibility to disease. If practical, outplanting should occur during the cooler months between October and May to reduce the risk of temperature stress and during calm weather to improve the chances of successful attachment.

- During transportation to outplant sites, corals should be kept in water. Avoid excessive heating and exposure to sunlight to reduce transportation stress.

- For each species, a minimum of 10 genotypes should be outplanted per site if feasible. A minimum of three replicate colonies per genotype should be outplanted at each site to increase the chances of survival.
- Outplanting of corals (both asexually and sexually produced) back to the natural environment may not occur at distances greater than 500 km from the location of origin since there is limited genetic transfer between populations separated by greater than 500 km.
- GPS coordinates and depth must be recorded for outplant locations. See the Monitoring and Reporting section below for additional recording information needs.

Monitoring and Reporting

Monitoring corals while in culture and after outplanting is important for determining coral health and success of population enhancement efforts. In addition, recording the origin and placement of corals is important for ensuring that outplanting efforts increase local genetic diversity. An adequate system should be in place to manage genotypes and lineages accurately during the processes of collection, nursery propagation, transportation, and outplanting.

This section describes recommendations for monitoring population enhancement that occurs during restoration and research. Any population enhancement that occurs as part of compensatory mitigation will likely have project-specific requirements that may not be the same as those discussed in this Management Plan. At a minimum, corals should be checked within one month after placement into a nursery. Loose colonies should be reattached and any high mortality noted. Subsequent monitoring needed for nursery maintenance and health determination should be conducted as appropriate. At a minimum, *in situ* nurseries should be checked semi-annually. *In situ* nurseries and outdoor *ex situ* nurseries should be checked immediately following any storm events.

After outplanting, initial monitoring of corals should occur within one month. Data collected may include the status (dead, alive, missing, broken) and condition (*e.g.*, amount of live tissue, amount of recent tissue loss, suspected cause of recent tissue loss, presence of bleaching/paling). Because culture and population enhancement efforts can include a large number of corals, monitoring all outplanted corals may not be feasible. Therefore, it might be more practical to choose a subset of colonies to monitor; a suggested subset is 20% or up to 50 colonies per site. Subsequent monitoring at least 6 months post-outplanting is strongly encouraged. Additionally, monitoring of spawning activity of colonies and genotypes should occur after outplanting.

Beyond colony level information, monitoring outplants at a larger landscape scale is valuable for evaluating the impact of population enhancement efforts. Longer-term monitoring is especially valuable since there is relatively little information available on performance of outplant sites beyond a year or two. Monitoring the footprint of area outplanted (*e.g.*, number of m^2 occupied by outplanted corals)

initially and how that footprint changes with time in subsequent monitoring efforts can be a useful measurement. There are multiple ways this type of information can be collected and evaluated. One way is photo mosaics that are created from overlapping videos or photos that can be stitched together (Gleason et al. 2007; Lirman et al. 2007). Photo mosaics provide a visual record of the reef, and changes in coral coverage and condition can be evaluated using subsequent photo mosaics (Griffin et al. 2015). Another method is swimming with a GPS around the perimeter of the outplanted area and taking waypoints at regular intervals. The waypoints can be displayed as a polygon in GIS. Subsequent swims around the perimeter of an outplanted area can be compared to see if corals have expanded beyond the original footprint (Walker et al. 2012). A third way is to monitor outplanted sites using techniques that have traditionally been used for wild coral populations such as transects, quadrats, or permanent plots. Measurements such as colony size (length, width, and height), density, or percent cover can be compared over time to evaluate population condition and dynamics.

For management of population enhancement efforts, information about outplanted corals should be submitted on an annual basis to NMFS, Southeast Regional Office (Alison.Moulding@noaa.gov). Essential information includes date, location, number of colonies and species outplanted, size of colonies outplanted, genetic information if available, and footprint of outplanted area (see Table 1 in Appendix A).

Risk Assessment and Management

The Policy Regarding Controlled Propagation of Species Listed under the Endangered Species Act lists several risks that should be evaluated in any population enhancement plan or population enhancement program. In this section, these risks will be evaluated based on the current level of knowledge. If incomplete or limited information exists, management strategies to reduce risks to wild populations outlined in the previous sections will be described.

> *Potential Risk 1: Removal of natural parental stock that may result in an increased risk of extinction by reducing the abundance of wild individuals and reducing genetic variability within naturally occurring populations.*

Because corals are colonial animals, staghorn and elkhorn colonies can be propagated by asexual methods (described above under 'propagation methods') without removing whole colonies. After an initial recovery phase, wild donor colonies have increased growth on the branches from which fragments are taken, thus indicating minimal harm to wild donor colonies (Lirman et al. 2010). The ability to use a small portion of a wild colony for propagation means that the abundance and genetic variability of natural populations will not be negatively impacted, and asexual propagation will not result in an increased risk of extinction. Furthermore, propagation within coral nurseries has led to preservation of genetic stock (Schopmeyer et al. 2012). Removal of a large portion of the donor colony could potentially increase the risk of colony mortality since smaller colonies are generally considered more susceptible to whole colony mortality (Hughes and Jackson 1985). However, guidelines in this

Management Plan regarding maximum percentage of a colony (20%) that can be removed during fragment collection have been developed to reduce the risk of donor colony mortality.

Sexual propagation is most often performed by collecting gametes from wild colonies without removing the colonies from their natural environment. Because fertilization rates in natural populations are likely to be low as evidenced by low sexual recruitment rates due, in part, to reduced proximity of compatible genotypes, removal of gametes is not expected to negatively affect natural populations. Therefore, regulated collections from wild populations are not likely to increase the rate of extinction or reduce natural population abundance or genetic variability.

Potential Risk 2: Equipment failures, human error, disease, and other potential catastrophic events that may cause the loss of some or all of the population being held or maintained in captivity or cultivation.

Catastrophic events are a potential threat to propagation efforts. *In situ* nurseries are subject to ambient water quality conditions such as temperature extremes and pollution that can negatively affect corals as well as other threats including disease outbreaks, predators, and physical damage from vessels, visitors, or extreme weather. Similarly, *ex situ* nurseries are vulnerable to equipment failure, human error, disease outbreaks, and extreme weather events such as hurricanes that can damage facilities and equipment.

The strategy in the Management Plan is to reduce the risk by incorporating both *in situ* and *ex situ* nurseries in a variety of locations and to replicate culture of each genotype in multiple nurseries so that impact to any one locality will not result in the complete loss of genetically distinct individuals. In addition, guidelines to reduce the risk of disease introduction and spread while corals are in culture (both *in situ* and *ex situ)* and after outplanting, including quarantine, health requirements for outplanting, removal of potential disease vectors, separate water treatment systems, and equipment cleaning, have been developed. *Ex situ* nurseries have the ability to be located anywhere on land where space is available and can house corals from multiple locations. Therefore, while *ex situ* nurseries are more complicated and expensive to run, it is beneficial to have several in various locations to reduce the risk of catastrophic events causing compete loss of some of the population. These can be used in combination with a variety of *in situ* nurseries that can maximize production due to less restrictive space requirements and lower costs.

Potential Risk 3: The potential for an increased level of inbreeding or other adverse genetic effects within populations that may result from the enhancement of only a portion of the gene pool.

The risk of inbreeding increases with smaller population sizes, lower genotypic diversity, and less connected populations. To reduce the risk of inbreeding, guidelines in this Management Plan recommend minimum numbers of genotypes per nursery to culture and outplant per site. Propagation of a genetically diverse stock can be easily achieved due to the ability to produce colonies asexually

using only a small fragment of the parental colony without reducing genetic diversity of natural populations. Outplanting a variety of genotypes at each location will increase the chances of successful fertilization by reducing the distance between genetically distinct individuals. Propagation through selective fertilization of gametes has a higher potential for enhancement of only a small proportion of the gene pool due to more limited collection ability from spatially separated genotype, but it also increases diversity by producing new, genetically distinct individuals to add to the population. Therefore, use of sexual and asexual propagation methods will reduce the potential for inbreeding by enhancing a higher proportion of the available gene pool and augmenting genetic diversity at outplanted sites to increase the chances of natural sexual reproduction.

Potential Risk 4: Potential erosion of genetic differences between populations as a result of mixed stock transfers or supplementation.

Based on the genetic information currently available, it appears that there is limited genetic transfer between populations separated by greater than 500 km and that there is little to no genetic exchange between the eastern and western Caribbean with the exception of Puerto Rico and the USVI. This east-west boundary is likely facilitated by water flow patterns coupled with larval dispersal potential. Management strategies are presented in this Management Plan to minimize risk of erosion of genetic differences between populations include limiting transfer of propagated colonies for outplanting and sexual crossing of gametes to less than 500 km from their source of origin.

Protecting genetic differences between populations is important if it represents some sort of local adaptation. There is potentially high adaptive variation in Florida populations of *A. cervicornis* that could be due to subtle environmental variation (Drury et al. 2016). Introducing as many genotypes as possible in nursery propagation and outplanting efforts, as recommended in this Management Plan, will enhance the frequency of adaptive genotypes and the subsequent rate of offspring survival and will help provide the diversity to cope with changing environmental conditions (Drury et al. 2016).

Potential Risk 5: Exposure to novel selection regimes in controlled environments that may diminish a listed species' natural capacity to survive and reproduce in the wild.

Corals are hosts to microbial communities and symbiotic algae called zooxanthellae, which together make up the coral "holobiont." Elkhorn and staghorn corals acquire these communities during early life stages such as during larval development or after settlement (Sharp et al. 2010). There is the potential for shifts in these microbial and algal communities over time (Little et al. 2004) and under differing environmental conditions. For instance, after bleaching, shifts in dominance of zooxanthellae clades have occurred in recovering corals, and corals moved from shallower or deeper environments have been known to change their density of zooxanthellae in response to changing light conditions (Toller et al. 2001). There is high variability in microbial communities both within and between corals (Daniels et al. 2011). *Acropora palmata*, and to a lesser extent *A. cervicornis*, appear to associate predominantly with one species of zooxanthellae (Baums et al. 2014), and the strongest difference between microbial

communities on Caribbean corals including staghorn and elkhorn corals is between host genera followed by between reef sites (Chu and Vollmer 2016). The importance and function of these microbial-host relationships are not fully understood. For instance, some microbes are thought to protect against disease (Ritchie 2006) while the proliferation of certain microbes may play a role in pathologic condition (Patterson et al. 2002). Shifts in microbial communities have been known to occur under stressful conditions such as high temperatures that cause coral bleaching (Ritchie 2006). Hundreds of microbes have been associated with diseased staghorn corals (Gignoux-Wolfsohn and Vollmer 2015).

There is the potential for shifts in microbial communities to occur when corals are moved between natural and captive environments. However, preliminary studies with other Caribbean coral species indicate that bacterial communities are similar between corals held in *ex situ* land-based facilities (both closed and flow-through systems) and those in the open ocean (Berzins et al. 2007), though this may not hold true in all circumstances (M. Miller, unpublished data). Therefore, while it is known that shifts in microbial communities occur, not enough information currently exists to determine the importance of these shifts or their impact on the species' ability to survive and reproduce.

Performance (e.g., survival, growth, reproduction) of individual genotypes is likely to vary in different environmental conditions such as those that may exist in both *in situ* and *ex situ* nurseries compared to the natural reef. Presumably, the more different nursery conditions are from the natural reef, the more likely there will be differences in performance. Asexually produced colonies outplanted from both *in situ* and *ex situ* nurseries have had high survival rates when monitored over short time periods (less than one year), indicating little immediate negative effect on short term survival, and staghorn corals derived from *in situ* nurseries were observed spawning two years post outplanting (Johnson et al. 2011). However, it is unknown if long term maintenance in controlled environments will result in acclimation to artificial conditions and diminished capacity for reproduction and long term survival in the wild, though the potential for temporal and spatial shifts in coral-associated microorganisms suggest capacity to adapt to changing conditions when introduced to more natural conditions present in the wild. In addition, access to microbial and zooxanthellae populations can be provided for early life stages of sexually propagated corals by maintaining them in local water (i.e., originating from the same state/territory or country as the gametes) or by keeping pieces of substrate conditioned in local water in the culture tanks. Therefore, the management strategies presented in this Management Plan that pursue establishment of both *in situ* and *ex situ* nurseries in combination with both sexual and asexual propagation methods will reduce risks associated with potential selection regimes.

> *Potential Risk 6: Genetic introgression (infiltration of the genes of one species into the gene pool of another through repeated backcrossing of an interspecific hybrid with one of its parents) which may diminish local adaptations of the naturally occurring population.*

Acropora prolifera is a naturally occurring hybrid between *A. cervicornis* and *A. palmata*, and hybridization can occur in either direction (*i.e.*, either species can contribute maternal DNA to the hybrid) (Vollmer and Palumbi 2002). Based on *Pax*-C intron data, Van Oppen et al. (2000) suggested that

backcrossing with both species occurs, but backcrossing was thought to be rare. Based on additional mitochondrial and nuclear DNA loci, Vollmer and Palumbi (2002) concluded that backcrossing only occurs between the hybrid *A. prolifera* and *A. cervicornis* and that the rates of genetic introgression from *A. palmata* to *A. cervicornis* are low. Limited rates of introgression suggest that the genetic impact on either parent species is low (Vollmer and Palumbi 2002). However, in some locations, such as highly localized reefs in Puerto Rico, Panama, and San Salvador, fine scale genetic structure was detected mostly due to the presence of introgressed genes (Vollmer and Palumbi 2007).

Guidelines in this Management Plan restrict crosses with the hybrid *A. prolifera* to research under controlled experiments. This will ensure that introgression occurring due to human manipulation is on a small scale under experimental conditions and is not occurring in the wild at higher than natural rates. This management strategy, coupled with the low rates of introgression observed in the wild, will minimize risk of diminished local adaptations of the naturally occurring population.

Potential Risk 7: Increased predation, competition for food, space, mates, or other factors that may displace naturally occurring individuals, or interfere with foraging, migratory, reproductive, or other essential behaviors.

After a motile larval stage, elkhorn and staghorn corals are sessile (attached to the sea floor) animals that sexually reproduce through broadcast spawning of gametes into the water column. They derive much of their nutrition from photosynthetic algae that live symbiotically in their tissue but are also filter feeders that eat plankton. They do not compete for food or mates. Outplanted corals will occupy benthic space, but this will not displace natural colonies since they do not actively seek space in their sessile state. Active selection of settlement location occurs in the larval stage, but population enhancement efforts are not anticipated to adversely affect settlement space as sexual recruitment rates are generally low and the space needed for settlement is on the scale of millimeters. Based on these characteristics of elkhorn and staghorn corals, population enhancement efforts will not displace naturally occurring individuals or interfere with any essential behaviors such as feeding or reproduction. This Management Plan recommends choosing outplant sites with low numbers of coral predators and suggests predator removal strategies to reduce predation pressure. Outplanted corals are not anticipated to increase predation on wild populations as predators of *Acropora* spp. feed on other species of coral, and presence of increased coral colonies may relieve predation pressure on wild colonies.

Potential Risk 8: Disease transmission.

Coral disease has been identified as one of the major causes of elkhorn and staghorn coral decline, and disease currently continues to affect natural populations. Detection of coral disease has largely been based on gross visual signs such as patterns and rate of tissue loss. Because the causative agent(s) of coral disease has been very difficult to determine, there currently are no screening tests that are able to

detect presence of disease before gross visual signs appear nor distinguish distinct disease conditions (*e.g*., more virulent from potentially more benign conditions). Thus, transmission of disease is a concern given the lack of understanding of disease etiology.

There are several best management practices identified in this plan that should reduce the chances of disease transmission. Fragments collected from donor colonies for asexual propagation should only be collected from branches without signs of active tissue loss as demonstrated by presence of stark white skeleton abutting live tissue. This is a minimum requirement, but if possible, collection from diseased colonies should be avoided all together. If new fragments are collected from the wild and placed in a nursery with existing fragments, they must be quarantined from existing colonies (placed down current on separate structures in *in situ* nurseries and placed in a separate water system for *ex situ* nurseries) for a minimum amount of time. Before colonies are outplanted, they must remain free of recent tissue loss and bleaching for a minimum amount of time prior to outplanting. Outplanting during very high (summer) or anomalously low temperatures should be avoided to reduce stress and potential susceptibility to disease. Outplant sites should not have environmental conditions (*e.g.*, sedimentation, reduced light) that could cause stress and potentially higher susceptibility to disease. Visible corallivorous predators such as the polychaete *H. carunculata* and the snail *C. abbreviata,* along with damselfish *S. planifrons and M. chrysurus* that create algal gardens by killing coral polyps, should be removed from nurseries to reduce potential disease vectors, and outplant sites with a low abundance of coral predators should be chosen. Finally, for *ex situ* nurseries, corals from different states/territories or countries must be maintained in separate seawater systems to reduce the chance of introducing novel microbe strains. While these actions may not prevent disease from occurring, they may help reduce the risk of disease transmission both within the nursery environment and to natural populations.

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Appendix A

Outplanted colony information for management and tracking of population enhancement efforts should be submitted annually to the Coral Recovery Coordinator at the National Marine Fisheries Service Southeast Regional Office. Table 1 can be used as a datasheet to submit the information (preferred in Excel format), and a description of the entry for each column is provided below the table.

Table 1. Outplant tracking and reporting datasheet.

Point of Contact	Sp	Yr	Date	Site Name	Depth (ft)	Lat. (DD)	Long. (DD)	# Col.	# Genotypes	ID Genotypes (*e.g.,* A (x20), B (x10))	# Corals per Size Class (*e.g.*, S (x10), M (x15))	Amount of Coral	Design	Total area (m2)	1 Mo. Monitoring (% survival)	6-12 Mo. Monitoring (% survival or total area)	Notes

Description of information to include in Table 1.

1. Point of Contact - Name of organization doing the outplanting.
2. Sp - Enter each species on its own row.
3. Yr - Year outplanting occurred.
4. Date - Date outplanting completed.
5. Site Name - If applicable, name or identifier of the site where outplanting occurred.
6. Depth - Depth in feet where outplanting occurred.
7. Lat. (DD) - Latitude in decimal degrees of site where outplanting occurred.
8. Long. (DD) - Longitude in decimal degrees where outplanting occurred.
9. # Col. – Total number of colonies of the species outplanted.
10. # Genotypes - If known, number of genotypes of the species outplanted.
11. ID Genotypes - Number of corals outplanted per each unique genotype. Record this as the unique genotype identifier and number of corals for each unique genotype in parentheses (*e.g.*, A (x2), B (x3)).
12. # Corals per Size Class – Number of corals outplanted per size class. Record this as size class identification (see below) and number of colonies for each size class in parentheses (*e.g.*, S (x10), M (x15)).
    - "X-small"= < 5 cm mean max diameter or up to 25 cm total linear extension (TLE)
    - "Small"= 5-15 cm mean max diameter or up to 100 cm TLE
    - "Medium"= 16-30 cm mean max diameter or up to 200 cm TLE
    - "Large"= > 30 cm max diameter or > 200 cm TLE
13. Amount of Coral – Multiply the number of colonies per size class by the midpoint of the size class (see below) and take the grand total for all colonies of that species outplanted. For example, for 10 Small and 15 Medium colonies, the entry would be 445 (calculated as (10x10)+(15x23)=445)
    - "X-small"= midpoint 3 cm diameter
    - "Small"= midpoint 10 cm diameter
    - "Medium"= midpoint 23 cm diameter
    - "Large"= midpoint 30 cm diameter
14. Design – Include attachment method, spacing, and arrangement of genotypes and colonies.
15. Total area (m^2) – Total area outplanted (the total "footprint" of the outplanted corals.)
16. 1 Mo. Monitoring (% survival) – % of colonies that survived through the 1 month monitoring event with > 0% live tissue.
17. 6-12 Mo. Monitoring (% survival or total area) – Enter either the % of colonies that survived through the 6-12 month monitoring event with > 0% live tissue or the measure of the total footprint of live *Acropora* corals in the outplanted area. Be sure to include units of measure (% for survival or m^2 for area).
18. Notes – Record any relevant information not captured elsewhere on the data sheet.

Appendix B

Updates to the Management Plan