

Acropora Coral Conservation/Restoration Workshop Final Report



12-13 November 2009
Smithsonian Institution, Washington, DC

Acropora Coral Conservation/Restoration Workshop

Final Report

<u>Section</u>	<u>Page Number</u>
1. Executive Summary	3
2. Asexual Propagation Considerations	6
3. Sexual Propagation Considerations	14
4. Health Issues Related to Outplanting	30
5. Genetic Considerations Related to Outplanting	36
6. Considerations in Forming a Consortium	39
Appendix 1 Beyond the Workshop	42
Appendix 2 Participant List	43
Appendix 3 Acknowledgements	47

Acropora Coral Conservation/Restoration Workshop

Final Report

November 12-13, 2009
Washington, DC

Executive Summary

Background

Coral reefs are some of the oldest and most diverse ecosystems on our planet that provide invaluable ecosystems benefits, nurseries and feeding grounds for fish and invertebrates, natural storm protection for coastlines, and potential sources for pharmaceuticals. *Acropora palmata* (Elkhorn coral) and *Acropora cervicornis* (Staghorn coral) are critical Caribbean reef-building species that once formed dense thickets and contributed substantially to accretion of reef habitats. As a result of multiple anthropogenic and natural impacts, these species have declined 80-99% from their historical population levels negatively impacting the structure and function of reefs throughout their range. Both *Acropora palmata* and *Acropora cervicornis* are listed as threatened under the United States Endangered Species Act (ESA), and are classified as Critically Endangered according to the global IUCN Red List.

It is recognized that coordinated management initiatives must be developed to address the multiple threats affecting these corals, protect remaining populations, and rebuild and recover degraded populations. A critical need exists to develop and disseminate both a best practices manual for asexual and sexual propagation techniques, and a strategy for managing health and genetic concerns for population restocking actions. The Smithsonian Institution and NOAA designed a workshop to take the first steps towards filling these gaps. The workshop organizers invited the Conservation Breeding Specialist Group (CBSG) – a Specialist Group of the IUCN’s Species Survival Commission – to facilitate the workshop. Using CBSG tools and processes designed specifically for this type of conservation problem, the **Acropora Coral Conservation/Restoration Workshop** brought together 42 conservation practitioners and subject experts from 5 countries to accomplish the following goals:

1. Identify the health and genetic concerns that stem from active propagation and population restocking (sexual or asexual).
2. Contribute to a collaborative “NOAA–approved” guidance document on the use of propagation techniques in recovery.
3. Develop a roadmap for creation of an out-planting plan.
4. Discuss the concept of forming a consortium of people doing active propagation of the species and engage stakeholders of recovery and conservation of Acropora.
5. Identify a working group to create a perspectives paper and those who would like to contribute to it.

Workshop Process

This intensive, 2 day workshop was conducted in Washington, DC from November 11-13, 2009. The workshop opened with welcomes from Alan Peters and Mary Hagedorn, representing the Smithsonian Institution's National Zoo, and Jennifer Moore from NOAA. The workshop facilitator, Onnie Byers (CBSG), reviewed the agenda and introduced the workshop process. This was followed by a series of presentations, summaries of which can be found below in this document. Mary Hagedorn and Valerie Paul (SI) hosted a preliminary *Acropora* coral conservation workshop held in Fort Pierce, Florida in February 2009. Jennifer Moore of NOAA Fisheries Protected Resources Division reviewed the Acroporid ESA Status and Recovery Plan. Tom Moore (NOAA Restoration Center) discussed asexual propagation in the US (ARRA-funded field nurseries); Dirk Peterson of Rotterdam Zoo presented recent information regarding sexual propagation; and Diego Lirman (University of Miami) reported on the latest international data regarding asexual propagation.

The rest of Day 1 of the workshop was dedicated to the task of outlining the potential content of a guidance document on the use of these propagation techniques in recovery. Two working groups (asexual propagation and sexual propagation) were formed to share lessons learned, discuss bottlenecks and their mitigation, and finally, to generate a list of best practices/guidance to be included in a manual. In addition, each group considered the question of the role of the *ex situ* effort in acroporid recovery. Participants self-selected into one of two working groups. Each group identified individuals to serve as working group facilitator (to keep the discussions focused and ensure that each person wanting to speak was heard), computer recorder (to keep track of group discussion on computer), and presenter (to deliver the working group report in plenary). Following the working group sessions, summary reports from the two groups were presented and discussed in plenary.

Day 2 was focused on health and genetic concerns to be dealt with in the outplanting phase of restocking strategies. Again, a series of presentations was given to ensure everyone was familiar with the basic concepts and the latest information on the subject. Jennifer Mickelberg from Smithsonian's National Zoo spoke about the conservation of small endangered populations, Iliana Baums from the Pennsylvania State University presented data on genetics concerns, Kim Richie from Mote Marine Laboratory discussed microbiological aspects, and Meaghan Johnson presented the findings of The Nature Conservancy's pilot out-planting study.

With this as background information, working groups were formed to focus on aspects of health and genetics that will need to be addressed in an out-planting strategy. Both groups were asked to identify all potential concerns, describe existing risk management protocols, define research needed to address these concerns and, finally, create a roadmap to an interim outplanting strategy. When they concluded their work, each group made a plenary presentation and their results were discussed to ensure that the output was understood and agreed upon by all workshop participants.

Outcomes and Next Steps

The *Acropora Coral Conservation/Restoration Workshop Report*, authored by all participants, presents the results of the efforts and energy they contributed to the workshop. Editing of the draft report was done using wiki technology with the assistance of workshop participants.

Outside review by non-participants was not part of the process. No content changes were made by the editors, and participants checked to ensure that accurate representations were made of their workshop products.

The detailed output of the workshop can be found in the individual working group reports that make up this document. To increase the potential for implementation, participants were asked to volunteer to commit to serving as champions for each recommendation. The major accomplishments include: the drafting of the contents for a best practices propagation manual, an outline of an outplanting strategy, and agreement to establish a coral propagation, recovery, and conservation consortium. In addition, consensus recommendations were made to pursue the establishment of an Acroporid species survival program or population management plan, and to draft a 'perspective paper' for journal publication describing the experience, expertise and passion of the *Acropora* restoration community.

Asexual Propagation Considerations

Goals

The overall goal for *Acropora* population restocking is to contribute to recovery of self-sustaining (i.e., successful sexual and asexual reproduction) populations of *Acropora cervicornis* and *Acropora palmata* composed of healthy colonies. One of the mechanisms to reaching this overall goal is propagation of corals via asexual reproduction. However, restoration of acroporids via asexual propagation is not solely for the purpose of mitigation: goals and expectations of recreating historical levels of reef frameworks are extremely unrealistic. A more realistic and achievable goal is to develop healthy, localized reef patches with capacity for successful sexual reproduction (capacity that is currently lacking in many areas). It is important to recognize that sexual reproduction is essential for preserving the genetic diversity of these corals, as sole reliance on asexual reproduction can result in genetic drift.

When conducting asexual propagation, several factors must be taken into consideration and criteria established in order to ensure successful outcomes. Important considerations and criteria in implementing any asexual propagation project may include, but not be limited to, the following:

- Proper **source population assessment** and identification of donor coral colonies (e.g., high genotypic diversity, fast growing genotypes, and/or a range of environmental conditions or habitats to which the donors may be adapted)
- Suitable **nursery site identification** and assessment (including physical considerations such as protection from high wave action where appropriate, presence of in-tact trophic structures, depth of nursery sites, as well as the presence of no-take area MPAs, etc)
- Determination and use of best **collection** methodologies (including considerations for size of fragments, disease prevalence, proximity to diseased colonies, etc)
- Determination of best **tracking** protocols (which may include the development of a number or lettering system to track genotypes from the original donor colony through successive generations of fragmentation to the outplanted colony/population)
- Determination of best **transport** and handling methods (such as maintaining fragments in cool temperatures and shaded from the sun, as well as utilizing dry transport methods and ensuring minimal physical damage).
- Determination, assessment, and use of best **planting and propagation techniques** (such as the use of rope and/or line methods, cement pyramid structures for *A. palmata* fragments, PVC frames, or use of cement, epoxy, and/or plastic cable ties for attachment to reef, etc)
- Selection of proper **management protocols for predation, disease and biofouling** such as algal competition (including the potential use of quarantine areas, using epoxy for mitigating the spread of diseases, and restricting the collection of fragments from diseased colonies)
- Determination of and use of best **long-term site maintenance** protocols (which includes considering the role of zoos and aquaria, trained volunteers, and the use of disposable nurseries as options for ensuring sustainability of nursery sites)

- Determination and use of best **long-term site monitoring** protocols (including maintaining measurements of light intensity, temperature, survivorship, mortality, growth rates, incidents of breakage, predation, etc)

With proper implementation of these considerations/criteria, one of the important objectives of asexual propagation can be met: to develop a healthy, genotypically diverse brood stock for the future purpose of outplanting coral fragments into wild populations. Developing a healthy and genetically diverse brood stock ensures minimal impact on wild donor colonies as well as preserves the genetic diversity of these coral species to allow for future re-population of degraded or extirpated coral reef habitats. Ultimately, restoration of acroporid corals via asexual propagation may prove essential to re-establishing the ecological health and integrity of Atlantic coral reef ecosystems.

Donor Site Assessment

The selection of appropriate sites for the collection of coral fragments from donor colonies for nursery propagation activities should be preceded by a comprehensive documentation of the status of the remnant *Acropora* populations within the larger project area, and a characterization of the benthic habitat and water quality for each potential donor reef. This documentation and characterization will provide direct input into regional efforts aimed at mapping the location and assessing the extent and condition of surviving populations of this threatened coral genus. Additionally, it will provide information on the correlative role that different processes (e.g., herbivory, predation, space competition) and physical factors (e.g., depth, light, flow) have on the resilience of *Acropora* populations.

The selection of donor site locations with surviving *Acropora* populations poses a challenge considering the widespread population decline experienced by this genus. Knowledge of historical ranges and habitat requirements can provide a good first step in the search for remnant populations. Potential sources of distribution information include: historical range maps, bathymetric charts, published literature and reports, and sightings reported by divers and fishermen. In places where extensive benthic monitoring exists (e.g., Puerto Rico, Continental US), digital benthic habitat maps can be an invaluable source of identifying potential donor sites.

Once a potential donor site with remaining *Acropora* colonies has been identified, the following information on physical and ecological attributes should be collected depending on time and resources available for a comprehensive site characterization:

Physical Attributes:

1. GPS location (of site and *Acropora* colonies or thickets if possible) and relationship to historical range and stands
2. Depth and depth profile/rugosity
3. Exposure level (e.g., exposed, sheltered)
4. Hydrodynamic attributes (e.g., tidal influence, margin location, proximity to known sources of stress factors like sedimentation and nutrients)
5. Level of management protection (e.g., MPA, research area, full access)
6. Water quality parameters (e.g., light penetration, sediment load, nutrients, DO, salinity, temperature, others)

7. Socioeconomic usage such as fisheries, tourism, etc. (relates to likelihood of long-term management/maintenance)

Ecological Attributes:

A number of very good rapid assessment protocols are available to assess the abundance and condition of coral reef organisms (e.g., AGRRA, ReefCheck). Acropora-targeted survey methodologies are described in Mayor et al (2006, Coral Reefs 25:239-242) and Williams et al (2006; <http://sero.nmfs.noaa.gov/pr/esa/acropora.htm>).

The choice of assessment methodology should be based on resources, time, and level of training, though should be reasonably comparable between sites. At a minimum, it is recommended that some estimate of abundance and condition (size, tissue condition, and prevalence of disease and bleaching) of surviving Acropora colonies be conducted. The following information should be collected for a comprehensive site characterization:

1. Cover, density, and/or sizes of key reef organisms (*Acropora*, other corals, sponges, macroalgae)
2. Condition of stony corals (mortality, prevalence of diseases and bleaching, macroalgal overgrowth, sponge overgrowth)
3. Abundance of coral predators and visible damage/indicators (fish, gastropods, worms)
4. Fish abundance, diversity, and size structure
5. Abundance of other key macrofauna (e.g., lobsters, octopus, grouper, moray, lionfish)
6. Grazing (urchin/fish), accumulations of sediment, presence of *Cliona*

Many of these metrics can be extracted from video or photographs that also provide a permanent visual record of site and colony status so it is highly recommended that scaled digital images of the benthos and Acropora colonies be obtained for each donor site. During the process of characterizing sites be sure to consider current research and approaches. For example when considering the health and of coral some work suggests that corals with disease can have healthy areas fragmented and multiple ramets of moribund colony genet be preserved. These corals might need to be kept separate initially from existing nurseries, but giving up or not using diseased colonies can result in a waste of a colony or genet.

Nursery Site Identification

Identification of suitable sites for a nursery is similar to donor site assessment described above. One primary consideration in choosing a nursery site is the appropriate level of protection from physical disturbances. Below are listed some other considerations in the nursery site identification that need expansion and greater detail as this work develops and the collective knowledge base continues to be generated.

1. Trophic structure needs to be considered with regard to suitable substrate, predation pressure, and other biological factors

2. Physical parameters need to be considered with regard to (at least) surge and temperature variation
3. Species-specific habitat requirements such as temperature, light, water motion,
4. Potential land-based sources of pollution
5. Site accessibility needs to be considered as it relates to materials transport, how often and what kind of activity will be done by practitioners, as well as invited and uninvited guests
6. Depth of coral in the water needs to be considered as it relates to growth of the coral and workability of the site
7. Growth rates observed in the area are a good indicator of site suitability. This may be assessed from coral currently growing on the site or test specimens placed in the area.
9. Nearby quarantine sites may be needed, but this is a topic requiring investigation. Are there areas that diseased coral can be moved to? How is a quarantine site defined? Does moving the coral away from primary nursery site actually remove the threat of spreading disease given the proximity to other coral and area currents?

Collection

The considerations that relate to the physical collecting of coral start with the method of collecting the coral. This topic needs more testing, documentation, and sharing of methods for optimization across a range of environments. Fragment lengths impact the survivorship: fragment lengths that range from 5 to 15 centimeters have been suggested with a potential greater success with shorter range sizes. Cutting pliers and carbide saw blades are the most commonly used tools.

Important data gaps need to begin to be filled: How do we ensure health of donor fragments? What is an appropriate minimum distance for donor colonies from obviously diseased colonies? The disease of most acroporids have not been shown to be contagious and the etiological agents have not been established but for limited cases. A conservative approach is suggested. Look for areas that currently have good recruitment of new coral growth and with no visual signs of disease.

Tracking

Keeping good records and tracking the corals is critical to managing and planning appropriate restocking design and up-front thought and effort should be allocated to this aspect. Existing methods have been developed using discs with a letter and number system to identify the original donor colony (hopefully genotyped), fragment generation, and individual fragment. Information regarding genotype, parent colony, location, photographs, performance history and site parameters can be tied to the fragment tracking number in a database. Various methods need to be tested for effectiveness in tracking. On-line databases, photography, and bar coding are all possible methods to enlist.

Transport

1. General Transportation Considerations
Corals can be transported very long distances as demonstrated by the tropical aquarium industry every day. Corals appear very resilient to handling and being removed from seawater but when something does go wrong, it can happen quickly. Most marine

biologists are accustomed to moving marine organisms submerged in water. Indeed this is critical for many. But as Ed Bronikowski discovered accidentally and reported in 1982, “waterless” or “dry transport” of corals may be superior to aquatic transport. It is certainly much less expensive to ship corals dry as the weight of bulk water is avoided, along with the possibility of seawater leaks. Heavy mucus production in injured or recovering corals also release a lot of mucus and provide substrate to heterotrophic bacteria that drop oxygen levels to the point where hypoxia and carbon changes normal microbiota can deleteriously stress or cause mortality.

Whichever method is selected, there is general agreement that coral exposure to direct sun, wind, physical impacts, and temperature extremes should be minimized or avoided altogether. Corals appear to handle slight cooling better than warming. And although corals have survived transport periods of 72 hours or more, transport times should be minimized especially for *A. palmata*.

There does not appear to be a consensus on the use of rubber gloves for handling corals. Indeed many do fine after direct, gentle human contact. Certainly one should avoid handling any animals directly if sunscreen, lotion, insect repellants, fuels, uncured epoxy, etc. is on one's hands. If they can't be thoroughly cleaned, then the use of gloves is recommended.

2. Wet Transport

When transferring live coral fragments relatively short distances (i.e. up to an hour) by boat, time of year (season), weather, and sea conditions should be considered: cool, calm, overcast days are best. Things to consider are size of fragments and to prevent any jostling. Even with calm seas, sloshing inside of transport containers can be significant on a boat. Fragments fewer than 30cm can be transferred in buckets, coolers or trays. The water should be changed frequently and monitored for temperature. Shading is critical: cover the containers, make sure the boat has a canopy, and/or transfer early morning or late evening to avoid heat and sun exposure. Use of a battery-operated aerator may help insure wet transport success. There should never be any cloudiness or haze appearing in the transport containers.

Larger fragments (30-140cm) have been transferred using a large sea-soaked foam (sponge) as cushioning on the boat floor, and the corals covered in sea-soaked sheets and towels, washed multiple times without detergent prior to use, and doused frequently with fresh sea water (per Harold Hudson's suggestion - this worked). The same works with smaller fragments, and unbleached or no chemical paper towels.

3. Dry Transport

“Dry transport” is a misnomer; the corals should be moist but not in bulk water. Some have speculated that corals may shut down and reduce metabolic rates with this transport method. Polyps are retracted and this may confer physical protection as well. It is also possible that with just a boundary layer of water on them, gas exchange is facilitated and hypoxic water avoided. During wet transport it has also been observed that if one coral dies and sloughs tissue, others usually follow leading to a “coral bouillabaisse”

(Bronikowski, 1982). Perhaps the dry method avoids the aqueous communication of stress factors that may be involved. Dry methods are suitable for both short and long transport times.

There are numerous variations on the dry method, but all basically involve briefly draining corals and wrapping each piece in wet, protective material. The following materials have been reported to work: newspaper, plastic strips, cloth towels and bubble wrap. Note that plastic tends to form a tight seal against local areas of coral tissue and may promote local hypoxia or anoxia and provides little in the way of keeping all surfaces damp. Plastic does not breathe and porous non-toxic material are better. Bronikowski (1982) successfully used nylon stockings filled with Perlite and found that synthetic sponges were not suitable.

4. Temporary Holding

Considerations of temporary storage of wild corals include duration and location of temporary holding can impact survivorship and changes in the future growth of coral. Considerations of the conditions at collecting, containers used in holding, methods of suspending the corals, and duration of holding need more exploration. Plasticized treatments of plastic containers have been known to cause gamete destruction and could impact wild fragments of coral.

Planting and Propagation Techniques

1. Fragment Culture

Field and in-aquarium techniques have developed and been shared mostly through word of mouth. For example, nurseries that grow coral on block bases that sit on the sea floor require lower energy environments to protect coral from the wave action. Growing coral suspended on lines requires more wave energy to aide in the health and growth of the coral. These techniques need more detailed written and video documentation to be useful to the broader working community. Technical experience like positioning coral fragments to maximize contact between substrate and healthy coral tissue encourages the attachment of the coral. If the coral is on the side multiple new axial corallites will increase growth rates. Planting in up right Christmas tree position results in slowest growth and attachment, and exposes cut edge area to potential invasion from substrate surface where the most native microbial flora and other potential parasites (ciliates, etc.) can gain access. This kind of information will be a significant portion of a manual when developed, needs to be described in much more detail, and compiled in one place.

2. Tissue Culture

Methods are in development for asexual reproduction based on micro-dissection of coral tissue for micro-propagation. The concept of these and other novel asexual propagation techniques need to be explored for both basic research and potential restoration applications. Tissue culture in other biological systems has provided a powerful tool for understanding physiology and maintaining cell lines over extended periods of time. This approach also has the potential to provide a large number of daughter colonies from a

very small amount of tissue, thus, minimizing impacts on wild coral populations. Associated R&D is addressing tissue cryopreservation with subsequent grow-out of propagules. Along with cryopreservation of sperm, and potentially eggs or embryos, this may provide another viable means of archival storage of the holobiont for eventual grow-out and colony restoration.

Management Protocols for Predation, Disease and Biofouling

Consideration of predation pressure and fouling of coral growth by colonization of other organisms is significant. It has been suggested that culturing coral on ropes helps minimize growth of other organisms by not providing as much surface area in close proximity for establishment of those organisms. Surfaces with small mesh size tend to encourage growth and should be avoided. Anti-fouling paints have been tried, but long-term consequences to the environment have not been tested, with zooanthallae reproduction being the first negative consequence to be considered. Routine schedules for manual cleaning surfaces and removing new recruits of sponges and other aggressive algae can minimize detrimental impacts to the coral.

Coral disease is an overarching consideration. Mitigations are being tested before the diseases are fully understood. Avoiding collecting diseased coral is the first line of defense, but disease can manifest itself following the collection event. Epoxy bands, copper wire, and removing diseased portions are just a few of the methods being used in trial cases. Growing coral on different substrates and methods also may impact disease. Some suggest clay bases and others favor line methods to reduce disease impacts. This is one of the areas that needs much more work to understand in order to tackle this hurdle of restoring coral.

Long-Term Site Maintenance

Long term maintenance of a nursery requires careful planning from the beginning. From the start, have a plan for closing down the operation so that materials will not clutter the site once the work is finished. Line nurseries may have less material than bottom nurseries to manage at the end of the work or if close-out process is initiated due to lack of resources to continue the work. The size, potential for expansion, and site limitations need to be part of the plan. What is the specific sites' optimal production level including the future outplanting? Logistics of how do the corals get to the nursery and how are the corals moved out is too easily overlooked, yet are crucial to nursery success. A production model needs to be tested. Staff and augmentation with volunteers and students need to be planned for in the initial business model.

Long-Term Site Monitoring

Workshop participants recognized that the monitoring efforts goals may be different for nursery production versus research. The monitoring efforts and goals may be tiered with production monitoring goals, common to most nurseries, as the first tier followed by more project specific research goals. The need for some consistency in monitoring goals, methods, and frequency was discussed by workshop participants.

Monitoring efforts may be divided into physical and biological parameters. The inclusion of the following list of parameters is dependent upon the resources (time, equipment, expertise, and funds) available to each nursery project.

Physical parameters at the nursery site:

1. Temperature – loggers that can record temperature at the nursery location over long periods of time (months to years) are easy to use and inexpensive
2. Light – these instruments are not as common but are available
3. Flow/currents – more difficult to measure and monitor and but general information on currents may be available for some nursery locations. Hobo-type loggers are also available, particularly for flexible nurseries.
4. Sea state/weather conditions – monitoring general sea state and weather conditions may prove useful when identifying causes of breakage and mortality as well as fish cleaning/predation and sedimentation/disease
5. General water quality parameters – salinity/fresh water pulses, chronic turbidity, nutrients, etc.

Biological parameters at the nursery site and (potentially) in the nursery itself:

1. Condition of the nursery site habitat (perhaps less important for nursery sites located in large sandy areas, or in mid-water or off-substrate nurseries)
2. Presence of predators – predator identification and abundance/density estimates, to include recruitment of predators directly to nursery

Biological parameters for the nursery fragments (per genet):

1. Survival
2. Partial mortality
3. Growth – this was a parameter that the workshop participants recognized as being problematic. There may be several ways to define and measure growth.
4. Disease
5. Bleaching
6. Predation
7. Nursery fouling a) organism(s), b) associated stress/bleaching/death

Not all of the parameters above need to be monitored at the same frequency. The frequency may also depend on the length of time since fragments were transplanted, fragment/coral size, or nursery methods/style chosen. Monitoring may be required more frequently (weekly) immediately following transplantation but less frequent (monthly) after the fragments appear to be stable in the nursery.

References

Bronikowski, Jr., Edward J., 1982. The collection, transportation, and maintenance of living coral. Ann. Conf. Amer. Assoc. of Zoological Parks and Aquariums. Phoenix, AZ. 5 pp.

Sexual Propagation Considerations

Spawning

Caribbean *Acropora* spp. are less predictable in their spawning times than other Caribbean species (notably *Montastraea* and *Diploria* spp), with spawning reported at various places across the Caribbean between nights 1 and 6 after late summer full moon(s). Spawning generally ranges from 120 to 200 minutes after sunset. The earliest recorded acroporid spawning observation in the Caribbean and the Florida Keys was on July 17 and the latest was on September 20. When the August full moon occurs early in the month of August or in late July, a split spawning event may occur with some colonies releasing gametes after the full moon in September. While *A. palmata* usually spawns annually, *A. cervicornis* may not (although historical data are missing). Spawn collection and larval rearing of this species therefore lags that of *A. palmata*. In some years (most recently 2008), the number of colonies or the percentage of a colony releasing gamete bundles is greatly reduced in *A. palmata* at some sites. Observations are slowly accumulating which suggest that, in some areas, individual genotypes spawn asynchronously (i.e. only one large genet may spawn on one night, Elbow Reef Florida Keys) or, in some robust populations, spawning is not observed on the predicted nights every year. For instance, *A. cervicornis* and *A. palmata* spawned at the Sea Aquarium reef in Curacao in 2007 on days 5 and 6 after the full moon, but failed to spawn during the predicted nights in 2009.

There is broad consensus within the group that a major hindrance to advancing sexual propagation as a broad scale tool in *Acropora* restoration is the limited number of sites where *Acropora* spawning and larval culture have been successfully carried out (primarily western populations of Puerto Morelos, Mexico; Carrie Bow Cay, Belize; to a lesser extent, the upper Florida Keys; and in the genetically ‘mixed zone’ of Puerto Rico). In particular, there is a paucity of data documenting where spawning populations exist across its range.

Generally, it was agreed that larval culture should be actively pursued in at least 2 or 3 sites in each genetic region (East and West) and at least one land-based experimental grow-out facility should be developed in each region to facilitate research. There are currently plans and funding in place to develop such a facility in Curacao, but none is yet slated in the Western province. Much of the successful larval culture and some spat outplanting work to date has occurred Puerto Rico but this is a genetic transition zone and so perhaps should not be a geographic priority. Such a ‘spreading the risk’ approach is needed, given the rarity of known spawning events and likelihood of stochastic inhibitors such as hurricanes. In short, investment is needed, first to make systematic observations of *Acropora* spawning, and then to develop needed infrastructure at sites where spawning is deemed reliable and logistically feasible. It is likely that local stakeholders could be involved in the documentation of reliable spawning by monitoring various populations, but this requires organization, persistence and commitment of those involved (to dive for 5 straight nights) and improved compilation of spawning reports from dispersed sites.

The required characteristics for a workable spawning site fall in three categories:

1. Ecological: Abundant, large, genotypically diverse colonies which spawn predictably and reliably in accessible locations and at sufficient density for fieldwork logistics.
2. Logistic: Reasonable shore-based facilities including adequate electrical power and

access to good seawater. Development of a deployable larval rearing kit is in progress for remote locations or boat-based efforts.

3. Bureaucratic: Access to permits, lack of taxes/duties on shipped gear, etc.

Unfortunately, few locations meet all these criteria. The table shows a compilation of the collective knowledge of the group and points to several high potential sites including Punta Cana, Dominican Republic (Western population), St. Thomas, US Virgin Islands (Eastern population), Antigua (Eastern population) and possibly additional areas in Belize (Western population). Additional research (possibly including focused histological study) is needed to better predict *Acropora* spawning times in Curacao as concerted observations over several years have shown these abundant, healthy, and genotypically diverse populations do not spawn profusely during the 1-6 day AFM predicted window. Histology in the months prior to spawning may also help identify potential sites throughout the range. It was also acknowledged that spawning and larval culture efforts should persist in certain sub-optimal locations, such as the Florida Keys, to compare spawning and larval success with more robust spawning sites.

Collection

One of the challenges of working with spawning corals is collecting the gametes that they release. Both species of Caribbean *Acropora* release eggs and sperm packed into pink bundles that are positively buoyant. In general, it is bad practice to bring acroporid corals into the lab for collecting their gametes. This is widely practiced with Pacific *Acropora* spp.; however, in the Caribbean, it is difficult to determine which coral colonies will spawn and collections of either species may not spawn at all. *Acropora palmata* is an especially sensitive species, and may not survive if brought into standing water for extended periods (> 48 hrs) of time. Even in ideal flowing seawater systems, maintaining acroporid fragments in aquaria can result in stress, patchy bleaching, or tissue loss within hours to days, leading to physiologically compromised corals and therefore constitutes a risky endeavor.

It is more feasible to collect *A. cervicornis* and *A. prolifera* fragments for spawning as long as they remain in the field most of the time. In Belize *A. prolifera* and *A. cervicornis* have been collected and moved to a shoreside location under a dock. Around sunset, corals are placed in individual buckets. If they spawn, gamete bundles are collected and used for fertilization. After the typical spawning window ends (200 min after sunset), these colonies are placed back under the dock. Spawn has been successfully collected with this method; however, it is often difficult to find fecund colonies without breaking many branches. We highly recommend collecting gamete bundles in the field and bringing them back to the boat or laboratory for fertilization and larval rearing.

Gamete bundles can be collected while they are in the water in multiple ways. One may try to suction the bundles up as they float to the surface; however, this is very labor intensive, ineffective, and often does not collect enough bundles for larval rearing on a large scale. Gametes can also be collected from the surface of the water above colonies that are spawning or from slicks that form as the bundles float and accumulate at the surface. While this means that you do not have to be in the water during spawning, it is impossible to determine which species of coral you have collected (or if you have collected hybrids), since many corals release gametes

at similar times during a night of spawning. Furthermore, bundles at the surface may be near the point of breaking apart and if nets are used, the bundles tend to break and the eggs and sperm are lost through the nets. Depending on the spawning event and the goals, it may be necessary to shuttle spawn from colony to shore numerous times during the entire spawning period.

By far the easiest method to collect large amounts of gametes is to use gamete nets. Gamete nets have been designed in numerous manners. Large static nets have been used to cover entire colonies. This method suffers from being unwieldy, water movement snagging or tearing nets on coral branches, and the distinct possibility of covering colonies that do not spawn at all (if they are deployed ahead of bundle setting). The other net types are variations on funnels, butterfly-like nets, or small fixed nets that cover individual branches. Each can be used successfully under various conditions or needs. The advantages of smaller static nets is the ability to identify colonies “setting” egg bundles and then quickly fixing nets over parts of the colony and returning once spawning is complete. They are less unwieldy than whole colony nets, can collect from individual colonies, and are more easily deployed, but still require some manipulation effort in terms of having to dive with buoyant floats that keep the nets erect over the branches.

Handheld butterfly nets are easily deployable and are the most effective for collecting spawn from numerous colonies. Divers can swim from colony to colony looking for bundle “setting” and then wait for release, collecting a batch of bundles, and moving quickly to other colonies. While possible to collect from individual colonies, the motile nature of this type of collection is less effective at ensuring individual or tagged colony-only collection, but also minimizes any abrasion or damage to nets and/or coral colonies. While net techniques requires significant amounts of time in the water during the nights of spawning, it also allows colony-specific collections (if working with specific genotypes or tagged corals, this is very important) and the collection of large amounts of eggs and sperm.

Here are a few different requirements for designing gamete nets:

1. Net material

There are several issues with net material. Mesh may be too large (bundles snag along the insides of the net from friction) or too fine (bundles are not pushed upwards into the collection bottle since the water does not easily move through the mesh). Rip stop nylon is a tough, smooth surface for the gametes to travel along and resists tearing in the field. If white nylon is used the gamete bundles are visible through the netting material. Construction should be with seams on the outside to minimize abrasion of bundles as they float into the collecting device. Finally, any folds of material along seams or near the neck should be avoided (fitting a funnel to the top helps) to minimize trapping bundles. Gentle wafting on the outside of the net, so that gamete bundles move away from the side of the net and move up into the cup may help. Further work is needed to determine optimal taper, shape, mesh size, fastening and/or framing for static nets, and transitions from net to collection bottle.

2. Net size

- Nets can be of a range of sizes, tailored to the size of the target colonies. Larger nets over larger colonies can collect more bundles. However, the taller the net, the more unwieldy the underwater handling, and the further the bundles have to travel to be captured in the cod-end, and many people experienced in gamete collection tend toward shorter tapers as being more effective. If there is surge, greater net length can be a hindrance to effective collection as the net can twist and pinch in the water, resulting in breaking of bundles. Nets should taper smoothly as they go up forming a cone. At the top of the cone, with static nets, a float is attached to keep the net upright during spawning. Alternatively, a small amount of air displaced in a collection bottle to achieve buoyancy.
- Mid-net support ring made of rigid airline tubing will keep the net from collapsing against the colony in strong currents. A sleeve can be sewn for the tubing at about two-thirds the height of the net to insert the tubing. The ends of the rigid airline are coupled with a small section of flexible airline and are ideally glued with silicone or hot glue for permanence.

3. Gamete cup/bottle (cod-end)

At the top of the cone, a funnel is attached to guide the gametes into a plastic jar. It is important that the mesh net be affixed to the funnel smoothly on the inside to prevent gametes from becoming stuck at the lip interface between funnel and net. Each jar can be numbered or color coded so that dives can keep track of which colony spawned into which jar. Numerous extra spawn collection bottles and lids should be available to divers and handling assistance (AKA 'bag ladies') are desirable to minimize loss of supplies or damage to colonies by the primary diver. The lid of a jar has a hole drilled through it and the funnel is glued or wired to the funnel so that the jar can easily be unscrewed and sealed underwater with an intact lid and then returned to the boat. Removal of the jar is tricky. It is important to calmly and slowly unscrew the cup while keeping it inverted. It is important to have the lid to the cup ready to screw on. Lids can be attached to the cup by gluing a string from the cup to the lid, or divers can carry extra lids.

4. Attachment to the corals

- Static nets may have a drawstring around the base so that it can be tightened over a coral or several branches. Additionally, the base of the static net can be fitted with weights (e.g. 'lead line') so that it can be simply placed over the colony (by far the easiest method).
- Tie-down ropes from the base of the net can also be helpful in heavy surge environment. Rigid plastic tubing can also be placed in a sleeve sewn into the bottom of the net to assist in keeping it open. These nets work well with *A. cervicornis* colonies because the hoop can be placed over colony branches with little securing to the benthos.

Field orientation dives and trial net deployment during the day is desirable to become familiar with handling and setting nets underwater. Navigating between colonies is much more difficult at

night. The use of transect lines, buoys, and/or cylume sticks is recommended. Setting nets is difficult while snorkeling (although possible on shallow reefs), and scuba diving is recommended. Always practice best night diving techniques, i.e. maintaining buddy pairs, avoiding deep settings, establishing a communication method between diving pairs, being familiar with the site, having multiple buddy teams to monitor as many colonies as possible. Task assignment is an integral part of the dive plan, such as determining in advance who is responsible for removing nets or swimming gamete containers to the boat. While diving, consider using signals (an underwater noise maker, glow sticks, a snorkeler who can communicate between groups) between buddy pairs to maximize the number of colonies searched and identify areas of the reef where the colonies are going to spawn. If individual colonies are to be collected or monitored, they should be marked before dusk with floats and glow sticks or other lights.

Approximately 15-60 minutes before corals spawn, they will begin to “set” bundles, becoming visible as pink swollen bulges in the polyp mouths. Generally, colonies will not have their tentacles out in a feeding response, although some colonies do spawn on some areas of the colony and not others. Once the divers have identified colonies that are setting, static nets can be set over that area. Nets should not be set on colonies that have not begun “setting,” as effort and nets can be wasted on colonies or branches that do not spawn that night. On shallow reefs, surge is often an issue during spawning, so ensure that nets are well attached or they will wash away. During spawning, periodic monitoring of nets will ensure that they are still covering the colony and gamete collecting cups have not fallen off of the net (if you do not have screw-top lids). It is helpful to have snorkeler(s) assist in such net monitoring. Additionally, snorkelers can quickly swim over many colonies to help identify colonies that have “set” and guide the diver to those colonies. If it is desired to collect data during spawning (colony number, species, set and spawn times), glow in the dark dive slates can be used.

If genotypes of colonies are not known, sketch a rough map of the colonies and approximate distances, so that the colonies that are further apart can be crossed. This will help reduce the likelihood of mixing clonemates. In general, very large colonies that have their attachment points on the actual seabed are less likely to be clonemates than smaller colonies nearby large colonies. These can be marked at night with a buoyant lighted float, or underwater with nails and/or tags. GPS waypoints can also be taken by snorkelers at the surface before spawning, and GPS can be used to locate specific colonies at night and for future monitoring.

After enough bundles have been collected, return them to the boat or the shore as soon as possible, preferably prior to when the bundles break up. A snorkeler or kayaker can be very advantageous to ferry gamete jars, allowing the divers to remain with the spawning colonies and nets. As individual buddy pairs of divers or “runners” transport collection bottles to the shore or boat, the collecting dive team removes the nets from the coral colonies. Nets on the colonies should be removed after spawning as they can inhibit water flow across the colonies, abrade the tissue, and can be caught in surge and will rip after excessive time on the colonies.

As the bundles break apart (typically 30-60 minutes but possibly as long as 90 minutes) after release from the colonies, the gametes are separated into eggs (pink particles floating on top) and

sperm (solution below, possibly milky white depending on concentration) and fertilization can commence. It is important to get collection cups capped and back to shore prior to bundles breaking up as sperm (which are not buoyant) will be lost. Excess water in collecting cups can be siphoned off to concentrate gametes in case bundles do break apart.

Further considerations are site- and project-specific. For example, cryopreservation requires gametes be returned from the colony as quickly as possible while they are still intact and highly viable. If there are tourists in the vicinity, make them aware of the ongoing activities and warn them not to interfere or remove the gamete nets, floats, or tags. Communication with local authorities helps to steer tourists away from the reef during a spawning event. Establishing communication networks with other researchers in the same area helps collaboration and communication for comparable results between different reefs.

One major research question is the variability of reproduction between genotypes across space and time. Genotyping the corals is a major bottleneck to answering this question; however, if multiple colonies are genotyped, it is good to tag them (color-coded cow or goat plastic tags are recommended as well as GPS waypoints in case tags are lost or removed) so that they can be monitored over different years of spawning. The tags can be numbered or color coded to mark individual genets and, depending on the weather, these same colonies could be monitored each year to determine variability in their spawning behavior. The Caribbean *Acropora* are quite variable in their spawning time and further research is needed to understand the physical and biological factors that might influence individual genet spawning behavior.

Fertilization

Background:

Successful fertilization in broadcast spawning marine organisms is dependent upon adult densities. If sperm concentrations are too low, fertilization will not occur. However, if sperm concentrations are too high it can lead to polyspermy, where more than one sperm penetrates an egg and leads to developmental failure. Fertilization dynamics are different for *A. palmata* and *A. cervicornis* eggs. In general, *A. palmata* eggs are difficult to fertilize, need 5-10 million sperm/ml to maximize fertilization; are much less compatible with *A. cervicornis* sperm than sperm from their own species; and self fertilize at low rates (< 4%) unless sperm concentrations are extremely high. *Acropora cervicornis* eggs require an order of magnitude less sperm to achieve fertilization; are equally compatible with *A. palmata* sperm as their own; and are more susceptible to polyspermy and self fertilization (avg. 20%). Polyspermic fertilization is seen in some crosses with *A. palmata* eggs but should not be of great concern unless sperm concentrations exceed 10 million sperm/ml. However, researchers need to be very cautious not to get sperm concentrations too high when attempting to fertilize *A. cervicornis* eggs. Self fertilized larvae are viable through settlement, but it is not well known if they are viable beyond this early stage. Yearling juveniles have been recovered in limited trials and it appears survival is possible even for newly settled larvae.

The number and size of eggs and number of sperm per bundle varies between *A. palmata* and *A. cervicornis* and within individuals of the same species. *A. palmata* has larger eggs and range from 3-11 eggs/bundle with a mean of 7 eggs/ bundle, while *A. cervicornis* has a greater range of

2-13 eggs/bundle, averaging 6 eggs/ bundle. *A. cervicornis* have more sperm per bundle ranging from 2-20 million/bundle, while *A. palmata* have 1-10 million/bundle.

How-to:

Generally, it is important to transport gamete bundles to the boat/shore prior to bundle breakup in order to maintain appropriate sperm concentrations for fertilization (sperm will be lost from open net cups in the water as they are not buoyant like the eggs, or will become too dilute in a lidded collection cup with few bundles). Successful fertilization can occur by adding a set volume of bundles from a mix of genets to a set volume of seawater. A rule of thumb is that each volume of bundles should be diluted with four volumes of seawater, and this should yield a 'lemonade' or 'watery milk' appearance to the resulting sperm solution. Again, it is best to err on the side of lower sperm concentration for *A. cervicornis* to minimize selfing and polyspermy. Be prepared with a range of sizes of graduated containers in case total spawn volume is more or less than anticipated (e.g., 15 ml and 50ml centrifuge tubes, graduated beakers or jars). This volume-adjusted mixture can be combined from multiple colonies/genets in a large 'batch culture' or fertilization can occur by separating the eggs and sperm (from the volume-adjusted stock from each parent) into separate stock and bathing the eggs with sperm from a different genet or a mixture of sperm from many genets. Gametes can be separated by pouring the mixture through a fine mesh sieve (~180 μ). The eggs are retained on the mesh and the cloudy solution caught below the mesh is your sperm solution. Eggs should be rinsed (using filtered seawater) at least 3 times with filtered seawater to ensure any sperm clinging to the egg's surface is dislodged. This method helps reduce the likelihood of self fertilization in *A. cervicornis*. If genets are known in the population, levels of genetic diversity in the resulting larvae should be optimized by mixing equal volumes of gametes from each parental genotype.

The length of time for fertilization depends on a number of factors. The sperm exposure time does not appear to be imperative if the proper concentration is used, and high (90%) fertilization has been achieved with time periods ranging from 15 minutes to 2 hrs. Sperm motility varies by colony, season, and other unknown variables, but under ideal conditions is extremely motile. Gentle swirling of the gamete solution is often employed to ensure higher fertilization. Alternatively, the high motility of sperm can still be effective in fertilizing eggs without shaking, provided that the eggs are in a container in a shallow layer, not more than 1-2 eggs deep. Thick layers of eggs in the fertilization container can result in low fertilization rates. Other factors to consider in the fertilization stage include maintaining dissolved oxygen levels (sperm have very high metabolism and can deplete oxygen levels fairly quickly) and, possibly, temperature.

Sperm becomes dramatically unviable if in contact with certain plasticizers; therefore, fertilization in glass vials is recommended. Near total sperm death has been observed in filtered seawater from new plastic water holding containers (specifically, grey Brute trashcans, although plasticizers from many plastics may have the same effect). It is also important to remove any small zooplankton from the collection bottles prior to fertilizations as they can consume bundles and eggs rapidly. A pipette using a focused light beam helps to remove plankton. Another method involves using a cooler, allowing the floating bundles to remain at the surface, and using a flashlight to attract plankton to the cooler drain, the drain plug opened, and the plankton flushed from the cooler.

Sperm should be rinsed out from (hopefully now-) fertilized eggs after a maximum of two hours to maintain good water quality for development. Usually, sperm solution can be siphoned from the bottom of the fertilization container and then filtered seawater added to rinse the embryos several times. Some have found that adding back a small bit of sperm solution to the embryos overnight may increase the fertilization rate.

There are differences in how gamete aging affects *A. palmata* and *A. cervicornis*. *A. palmata* eggs seem more likely to be affected by age. However, fertilization can remain high within 2 hours after gamete bundles dissipate. Fertilization drops significantly when *A. palmata* gametes have aged four hours, however the drop is less severe in *A. cervicornis* with at least 40% of the eggs remaining viable. Sperm motility decreases rapidly over time, and sperm should be used for fertilization as quickly as possible post-collection. One thing to consider, although it has not been confirmed in *Acropora*, is that in *Montastraea* spp. fertilization does not occur within the first 30 minutes after gamete bundles dissipate – but does in *diploria strigosa* from the Flower Garden banks.

Great variation is seen in degree of fertilization success between nights, sites, and years, and (aside from the need for multiple genets) the mechanisms for this variation are not understood. Potential mechanisms such as variation in sperm motility, specific genet compatibility, or environmental conditions need to be investigated as high fertilization success is a major determining factor in the success of larval culture overall.

If there is access to a microscope, embryos can be examined 2-6 hrs post fertilization to estimate fertilization rate. After 2 hrs, fertilized eggs will have begun initial cleavages and be in various states of development, whereas after 6-8 hrs you will see the ‘corn-flake’ stage (in contrast to the round plump eggs which are unfertilized). Following the cornflake stage, embryos begin to reform into rounded embryos and undergo gastrulation to become swimming larvae within about 48 hours. Fertilization and developmental success can be estimated as the ratio of these stages to the total number of eggs in an aliquot of the culture (e.g. a pipette squirt or a field of view on your microscope).

The positively buoyant embryos tend to clump together, causing an unfavorable microclimate. Additionally, the unfertilized eggs, and any untended dying embryos, will break apart, releasing positively buoyant lipids that can cause additional mortality and low microclimate oxygen. The water flow in developmental chambers must initially be quite mild to prevent shearing of cells actively dividing, but not too slow to keep them separated. Occasional hand stirring with a glass rod or a pipette connected to an air pump to produce a slow air bubble flow under clumped embryos may be needed to keep them separated and limit mortality. As development progresses past the cornflake stage, the larvae are capable of withstanding higher flow speeds.

Rearing Larvae

The most important factors in conducting successful larval-phase culture are:

1. Starting with high fertilization rates so that there is less egg biomass breaking down in the cultures
2. Keeping the larvae at moderate density and in gentle motion
3. Maintaining the cultures in good water quality at appropriate temperature to minimize stress.

Recommended water quality parameters are between 8.0-8.2pH, dissolved oxygen at least 80%, salinity 35-37psu, and temperature between 26-30 °C, ideally 28-29°C. There may be a range of appropriate water temperatures. Experimental studies with *A. palmata* in Puerto Rico show substantially reduced survivorship at 30 °C (Randall & Szmant 2009, Baums unpublished).

However, other groups have had success in raising larvae at higher ambient temperatures (e.g., 31 °C in Belize, Fogarty & Ritson-Williams, pers. comm., 30 °C SECORE in Puerto Rico 2007-2008). It is unclear if there is geographic variation in the optimal temperature for *Acropora* larval cultures. Generally, larval cultures should be maintained as close to 29 °C as possible. Cooler temperatures will result in slower development time.

During development, it is critical to maintain high water quality. This can be achieved through the use of flowing seawater and kreisels, water changes using filtered seawater in static bins, or a drip-type flowing replenishment. The initial 12 hours are the most sensitive period of development and high mortality can occur, even with healthy embryos, through fouling of water. There is some disagreement about the best time to place the larvae into kreisels. During the first 8-10 hrs after fertilization (through the 'corn flake' stage), the larvae are in their most delicate developmental stages, and it may be advisable to keep the larvae in standing buckets (with gentle bubbling) for the first night prior to placing them in the kreisels or else some shearing of the early phase embryos can occur. (Often these fragments can undergo apparently normal development, but the result is a culture with a wide range of sizes and, seemingly, many smaller settlers). Otherwise, kresiel flow should be at a slow rate with periodic gentle manual dispersal of embryos that clump together using a glass stir rod or a pipette attached to an air pump with airline to create a gentle slow bubbling under the larval aggregation. If there are concerns about fertilization success, it may also be beneficial to wait a few hours to verify that fertilization rate is reasonably high before committing them to kreisels or other culture vessels. This is more important in recirculating systems where water quality is an issue. Also of greater importance in a closed system is the maintaining a stable salinity of 35-37ppt.

If using kresiels, it is important to keep the bottom outflow screen cleaned from lipids and cell debris to ensure flushing of the kresiel and keep proper water flow and water levels in the vessel. If necessary, the developing embryos can be removed from the culture vessel gently in a sieve, rinsed in filtered seawater, and then put back into a cleaned development vessel. In addition, water quality should be monitored in the development vessels to ensure that temperature and water quality are optimal (28-29C, 35-37psu, 100% O₂ saturation, pH 8.0-8.2) for development.

If there are fertilization batches of different fertilization rates, the high fertilization batches should not be combined with low fertilization batches, and ideally should be kept in separate rearing systems. The amount of time it takes to try and save vessels with low fertilization in terms of efforts to control disintegrating unfertilized eggs generally results in very low larval survival, and most effort should be allocated strategically.

After 48-72 hours, the larvae will begin swimming. At this point, they should be removed from the culture vessel or they will begin to settle. It was suggested that settlement material can be placed into the rearing container directly to achieve settlement, but SCORE uses special settlement tiles to increase settlement success and larvae should be removed to avoid settlement in the rearing container.

If recirculating or flow-through seawater is not available, larvae can be reared in standing (static) containers such as bins or coolers. Round or oval containers are better than square ones to minimize the accumulation of embryos in corners. Gentle bubbling and/or manual agitation (e.g., with wash bottle down the walls of the container) is helpful. This approach is more labor intensive as frequent water changes with filtered seawater, possible sieve rinsing of larvae, and cleaning of the bins while cleaning developing embryos will be needed. Stable temperature of both static bins and exchanged water are a must.

Tools such as sieves (~ 40-180 μm for *Acropora* larvae), squirt bottles, and ‘gravy boats’ (i.e. pitchers that pour from the bottom so that buoyant larvae can be rinsed and the “dirty” water can be poured off from below) are very useful in changing water. In either static bins or kreisels, cultures often display a ‘crash’ phase (12-24 hrs post fertilization) during the time that unfertilized eggs are breaking down. During this phase, virtually constant water changes or cleaning out/scrubbing of kreisels is needed to prevent mass loss of otherwise healthy developing embryos.

Cultures are generally more stable after 2-3 days following gastrulation. Once the larvae in kreisels begin swimming (~ day 3), they should be watched very carefully when large numbers begin swimming to the bottom, they can clog the mesh and ‘swamp’ the kreisel. It is probably better to move the larvae to static bins or transport containers after they are swimming, though it may be a couple days before the majority are ready to settle, so several days of water changes would be needed. They can be transported at this (swimming) stage.

Settlement

It is clear that competent *Acropora* larvae will settle under a wide variety of conditions and on a wide variety of surfaces. While certain rare crustose coralline algae (notably *Titanoderma prototypum* and *Hydrolithon boergesenii*) will enhance settlement and survivorship of larvae, most common CCA are not friendly to new settlers, either overgrowing or sloughing them. While the larvae will readily settle on CCA, post-settlement survival is almost certainly lowered when compared to a clean settlement substratum. Some degree of ‘conditioning’ of substrates (soaking them in the field) seems to make them more attractive to the larvae, but may not be necessary. Larvae seem to undergo reverse metamorphosis or fail to undergo metamorphosis for

up to 30 days on unconditioned substrate using 0.22micron filtered seawater, compared to 1-3 days with conditioned substrate, indicating the importance of biofilms in inducing settlement and metamorphosis (Borneman, in progress)

Several recommendations confirmed by multiple groups included the importance of:

1. Maintaining larvae in good water conditions for as long as possible during settlement allows more larvae to reach a developmental window of competence. This can be facilitated by using fresh seawater or conducting settlement within kreisels or other vessels with drip input (rather than static vessels) in which several batches of substrates can be settled over time.
2. The settlement substrate should be left in the containers for several days to a week to allow the maximum number of larvae to settle (not all larvae settle immediately)
3. Proper salinity and temperature must be maintained during settlement
4. Substrate texture or roughness is an important consideration to facilitate settlement
5. Reporting settlement rates is better in terms of gestational age rather than time since exposure to settlement substrates. However, because of the many factors that could play into mortality at each stage post-collection, getting settlement rates from the number of competent viable larvae is still important.
6. Most experiments have been conducted when the larvae are 5-7 days old (after fertilization). Waiting longer increases the risk of significant mortality in larval cultures.
7. Light is an important factor in influencing the position of settlers as brighter light may increase their tendency to settle on under-surfaces

The settlement stage remains poorly understood, clearly indicating that research is still needed to optimize this step. A very basic question remains: What constitutes a 'good' settlement rate? There is no way to know what settlement rates are achieved in nature. The SECORE group seems to consistently achieve settlement in a range of 10-15% after long-distance transportation (> 24hr). The Smithsonian group in Belize has gotten settlement rates close to 80% (7-day old *A. palmata* larvae in the presence of *H. boergesenii*) but typically gets settlement rates between 10 and 30% in relatively small larval batches. Low settlement rates are an obvious bottleneck to recruitment, but may be sufficient when the initial number of larvae is high and is still many magnitudes of order higher than best estimates of larval survival in the field.

Scoring of settlement rates has been inconsistent among research groups. Replicated settlement assays are generally scored either 24 or 48 hrs after exposure to the substrata, but larger scale settlement observations suggest that many initial 'settlers' disappear within a couple of days. Overall, it is recommended to leave substrates and settlers undisturbed for several days to a week, and then score fully metamorphosed polyps. The SECORE group has been doing counts at intervals of 1 week, 2 weeks, 1 month, 3 months, 6 months, and 1 year.

Other general research questions and comments include:

1. What is the optimum density of settlers on substrates? Is there density dependent survivorship? Do individual primary polyps fuse and increase survivorship and should this be encouraged? Rinkevich et al. suggest immunological competence at approximately 6 months for *Stylophora pistillata*.

2. It is beneficial to include grazers (e.g., snails or hermit crabs) during the settlement phase since sediments and algal/cyanobacterial filaments, sponges, *Aptasia*, and virtually any other biota is competitive and deleterious to the growth and survival of primary polyps and very small colonies. Even coralline algae can overgrow these initial stages. However, aggressive or larger grazers can consume small developing colonies and primary polyps.
3. What are the best means of achieving zooxanthellae infection with the 'native zooxanthellate strains? To date, the SECORE group has been using semi-cleaned zooxanthellae from tissue blastate to inoculate settlers 2-3 days post settlement. The water level in the settlement containers is dropped to just cover the settlers and the zooxanthellae introduced for several hours to maintain a relatively high concentration for uptake. The water is then brought back up with filtered or clean aged seawater. Nonetheless, SECORE has shown that settlers are not specific in their choice of zooxanthellae and will take up and maintain non-native strains simply by exposure to the seawater of established closed coral reef systems. This is not, however, deemed to be a practice to be encouraged and must be avoided for any potential stock for reintroductions. In no case has acquisition of zooxanthellae been deemed to be problematic or limiting. There is, however, a window of opportunity for maximum zooxanthellae uptake that has not been clearly delimited, and recent evidence suggests that inoculation of zooxanthellae can be acquired before settlement in the swimming larval phase. Infection is not usually visually obvious for several weeks after metamorphosis
4. What settlement cues might increase settlement and initial survivorship in bulk cultures? Red and orange showed significantly higher settlement rates than other colors, and the settlement material is also highly significant, with acrylic highly preferred over limestone and ceramic. In addition, the lack of porosity of acrylic facilitated removal of sediments and establishment of filamentous algae and cyanobacteria as competitors in primary polyps and early colony growth (Borneman, in progress). The use of plastic in restoration efforts, however, is potentially problematic although colonies over 1 year in age will have completely embedded such settlement material within their skeletons. (Plastics and other related materials may be problematic for reasons related to release of toxicants such as plasticizers.)

The approach outlined is a practical approach; consideration in the context of research needs may be giving to approaches used in developmental cell biology, biochemistry and cell signaling to identify cell signaling pathways and other related processes to more clearly define the normal developmental process from a biochemical and cell biology perspective.

Post-settlement

Post settlement aquarium grow-out entails the use of a stable culture system that has been run for at least a few weeks to months and has passed through algal successions (especially Foraminiferes) which usually appear when a new system is started. Highly mature systems may, conversely, have too much diversity and encourage competitive colonization with *Aptasia* anemones, hydroids, sponges, and diverse algae.

The most successful example of a closed system culture set up has been that of Mitch Carl, Omaha Zoo. The grow-out system consists of two 7x3x0.5m stacked fiberglass troughs. The top trough drains through 2 two-inch drains that empty into the bottom troughs, also with 2 two-inch drains. Each trough has six return lines plumbed through the bottom of the trough and each is controlled by a valve. Each return has a 3/4" inch educator on it to maximize water flow. All returns and educators are pointed toward the same end of the tank with the drains. This creates a strong current that circles the tank. The pump that runs both troughs is a Sequence Hammerhead pump. Both troughs drain to a common sump that has some pieces of live rock in it for biofiltration. The only additional filtration is an ASM G4 protein skimmer. Calcium and alkalinity is supplied by a Knop calcium reactor. Lighting is provided by two 400 watt metal halide lights with Sunmaster Cool Deluxe bulbs. Lighting is very important, but doesn't have to be as high as previously believed or equivalent to the irradiance received in the field. Lights are lowered to about 6-8 inches off the surface (PAR: 200-300 $\mu\text{E m}^2 \text{sec}^{-1}$ at various parts of the tank), higher light levels might not be necessary and might increase algal growth.

Other important factors include:

1. Control of algal growth is essential: low nutrient levels, eliminate phosphates (e.g. Phosban, Rowaphos, Antiphos), algae grazers (blue-legged hermit crabs, *Cerith* snails or *Astraea* snails are useful and easily available). The use of small grazers rather than large ones (no sea urchins!) and surgeon fish prevent algae without damaging the juvenile colonies. Some manual algae removal may be necessary (small toothbrush or tweezers)
2. Control of other competitors: *Aptasia* (Four-eyed, raccoon, or copperband butterflyfish), sponges (manual removal), hydroids (manual removal)
3. Avoid sedimentation on settlement substrates: Settlers are ideally placed on an elevated platform above bottom; a sand bottom is optional and should be avoided if it causes sedimentation
4. Feeding: Mitch is using 5 capfuls DT's phytoplankton, 5 cubes of frozen rotifers, dime size amount of frozen oyster eggs, and a pinch of Golden Pearls. Shedd Aquarium has used all live foods with good success. These foods include a combination of artemia naupli, *Eutrepina* copepods, and rotifers. Feeding has been shown to dramatically increase the growth of corals (up to 3600 *Artemia* nauplii/l). Feeding is also essential prior to the uptake of zooxanthellae.
5. Maintain high water quality by monitoring water parameters and frequent water exchange as needed
6. Identification of early settlers can be facilitated by using NightSea goggles and flashlight from www.nightsea.com. However, not all settlers might show fluorescence!

Research questions:

- Determine ideal feeding regime
- Protocol for infection with zoox

Transport

What kind of material is transported?

1. Gametes
2. Larvae
3. Primary polyps / early recruits

Details:

1. Gametes

Gametes have a very limited life span and therefore must be handled quickly to avoid loss. Usually gametes start dying a few hours (sperm) to a day (eggs) after being released unless they are cryopreserved.

- a. Fresh gametes: during spawning event from collection site to shore or lab; collection during coral spawning nights needs good coordination between the different dive teams in the water and the team on shore. Onshore communications and needs should be well known prior to the spawning event. One person (coordinator) should be in charge to oversee handling of samples between divers and shore team. Within a dive team, it must be clear before the actual diving how samples are brought to the surface (dive safety first!). A kayak or snorkeler may help to move quickly between the different dive teams at a dive site for collecting samples and for bringing those samples to the shore team. Samples must be secured well against leakage and loss and appropriately labeled (use high quality HPPE [i.e. Nalgene] plastic bottles and collection bags to avoid losing sample bottles and to prevent any plastic toxicity). See also “working doc COLLECTION”.
- b. Cryopreserved samples must be kept frozen (-180 °C; liquid nitrogen) at all times and must be transported in dry shippers charged with liquid nitrogen. Dry shippers are the only transport device allowed on an airplane and are a safe method to avoid any accidents through direct exposure with liquid nitrogen. **IN ORDER TO AVOID ANY ACCIDENTS, YOU MUST READ THE INSTRUCTION MANUAL CAREFULLY!** (A manual should be provided with the dry shipper). The SECORE group routinely has used dry shippers in intra- and intercontinental flights without any problems as checked luggage (contact your airline in advance to avoid any delay at the airport!). Make sure that the dry shipper is charged in advance if liquid nitrogen is not available at the field site. Duration for a charge with nitrogen to maintain temperature depends on the rating of the particular shipper, but 2 weeks is typical. In tropical climates, effective duration may be somewhat less than the rating. Dry shippers can be rented from companies which trade with gasses.

Research questions: a standardized cryopreservation protocol is needed which can be easily applied by field workers

2. Larvae

Transportation of larvae is a very effective and successful way to transfer large quantities from the field over large distances. Transport volume and weight is relatively small compared to the transport of fragments of colonies.

Larvae of broadcast spawners, such as *A. palmata* and *A. cervicornis*, can be transported long distances (< 48 hr) when very clean, filtered sea water (0.5-1 µm) is used, and larvae concentration does not exceed 4,000 larvae per liter (Petersen et al., 2005). Any contamination with decaying material may lead to unnecessary bacteria growth during transportation and may lead to reduced larval fitness and survival. Use transportation bottles made from HDPE plastic with a wide bottleneck to avoid plastic toxicity, and place bottles horizontally in the transport box to avoid positively buoyant larvae from gathering in the bottle's neck which may lead to total loss from anoxia of concentrated larvae. Fill bottles completely with water; a small gas bubble may stay in the bottle. Aeration of transport water prior to packing with pure oxygen might increase survival; however, this still needs to be verified. A pilot study suggests that seawater filtration may be the most important factor.

The use of well-insulated transport boxes will avoid high temperature fluctuations during transportation. It is important that Styrofoam boxes are intact and the lids taped. Any break in a complete seal can result in dramatic temperature fluctuations during transport and heat or cold packs may be needed, depending on the transportation destination and duration. These should be taped to the underside of the Styrofoam container lid. Prior to field work, check logistics as to what is the best way to transport larvae – personally as checked luggage, as cargo, or by express service (FedEx, UPS, etc.). National regulations of both export and import countries must be clearly known in advance and necessary permits must be in place (CITES, collection permit, letter of origin; in exceptional cases, a health certificate might be necessary). Also, regulations for transportation of liquids on air carriers should be known in advance.

At the destination, the larval bottle water should be tested and parameters recorded. Ideally, this includes dissolved oxygen, pH, temperature, salinity, ammonia, nitrite, nitrate and phosphate (before packing larvae and immediately following completed transport). Larvae suspension should be acclimated slowly to new conditions. The condition of each transport bottle is monitored and recorded (mobility of larvae, clarity of water clear, smell of water) and 'low quality' suboptimal bottles should be kept separate from the others. Mobile larvae are not necessarily competent larvae and correlating transport conditions and water quality vs. post-transport settlement competency may identify potential settlement or survival issues. This will also help long-term data to optimize the transportation of larvae. Analyses have shown that especially oxygen and pH may drastically drop during transportation and higher concentrations of ammonium and nitrite have been observed.

Research questions: optimize water quality during transportation, especially pH, oxygen!

Reference

Petersen, D., Hatta, M., Laterveer, M., Van Bergen, D. 2005. Ex situ transportation of coral larvae for research, conservation and aquaculture. *Coral Reefs* 24:510-513.

3. Primary polyps / early recruits

Primary polyps and early recruits can be transported in larger quantities from the field to the lab/aquarium as well as between institutions. When settling larvae, it should be considered whether the settlers will be transported. If transportation is desired, the settlement substratum should be designed in a way to enable easy shipment. The SECORE group is using small tiles of a few centimeters in size to produce tile-recruit units which are then placed in a structure (plastic egg crate) to stabilize substrates from tumbling during transportation (Petersen et al., 2005, 2006). Two transports of more than 300 recruits of *A. palmata* carried out by the Omaha's Henry Doorly Zoo to the Rotterdam Zoo (duration from packing to unpacking approx. 48 hr) led to 100% post-transport survival (Carl and Petersen, unpublished data). The recruit-tile unit is hereby treated similarly to an adult coral, therefore shipped submerged in seawater with pure oxygen using double plastic bags. It is important to avoid any perforation of the bags, i.e. due to sharp edges of the substrate. Similar to transporting larvae, packing should ensure temperature stays as constant as possible and double-check with authorities and airlines on permits and regulations required. Ideally, transport boxes should contain a temperature logger. Likewise, water quality parameters should be measured and recorded prior to and following transport.

Research questions: can primary polyps / early recruits be transported dry in a moisture environment? *Montastraea faveolata* primary polyps have been successfully shipped overnight wrapped in moist washcloth so it is likely this would be successful with *Acropora* as well.

References

- Petersen, D., Laterveer, M., Van Bergen, D., Hatta, H., Hebbinghaus, R., Janse, M., Jones, R., Richter, U., Ziegler, T., Visser, G., Schuhmacher, H. 2006. The application of sexual coral recruits for sustainable management of ex situ populations in public aquariums - SECORE-Project. *Aquatic Conservation: Marine and Freshwater Ecosystems* 16(2):167-179.
- Petersen, D., Laterveer, M., Schuhmacher, H. 2005. Innovative substrate tiles to spatially control larvae settlement in coral culture. *Marine Biology* 146(5): 937 – 942.

IMPORTANT: DO NOT REINTRODUCE ANY CORAL MATERIAL FROM A DIFFERENT REGION INTO THE FIELD! ANY MATERIAL THAT HAS BEEN TRANSPORTED AWAY FROM A FIELD LOCATION MUST NOT BE USED FOR ANY REINTRODUCTION ACTIVITIES UNLESS THERE ARE ADEQUATE RISK-MANAGEMENT PROTOCOLS AVAILABLE AND IMPLEMENTED!

Health Issues Related to Outplanting

Goal and Summary Recommendations

What are the goals of outplanting?

Establish self-sustaining, genetically diverse, sexually reproductive pockets of Acroporids that are able to establish and expand to a given area of reef.

Summary Recommendations

- Propagation of transplants falls under a continuum of culture platforms ranging from field nurseries through shore-based flow through tank culture to inland closed systems. The health risks associated with different culture platforms follow a similar continuum from lower risk platforms that are closer to the natural reef to higher risk platforms that are farther removed. Generally, transplants should be sourced from the platform representing the lowest risk available. For stocks reared in higher risk systems, techniques and practices should be devised through applied research for targeted risk reduction.
- Only healthy organisms should be used in outplants. However, our capacity to discern what is a healthy coral fragment is extremely limited. Thus, we should exercise common sense in applying the currently available crude health assessments while actively monitoring and evaluating the health/performance of out-plants and actively pursuing more precise criteria for assessing coral health in concert with improved and tractable diagnostic tools.
- Outplant material should be drawn from sources utilizing good husbandry practices (clearly defined in a guidance document) and this should be an aspect of permitting/regulation.
- A wide range of characteristics need to be considered in selecting appropriate sites for out-plants, including environmental conditions (e.g., sedimentation, wave exposure), ecosystem or trophic integrity (perhaps related to MPA status), historical occupation of the site, and site value (e.g., in terms of tourism, fish habitat, transport of potential larval production, etc.). While it is logical to prioritize ‘good’ sites in initial phases of wide-scale outplanting, sub-optimal sites should not be neglected as they may be able to host differently-adapted genotypes that may be important in maintaining the full range of genetic diversity.
- Highest research priorities include 1) Development of sensitive, specific and cost-effective diagnostic tools to determine health status; 2) Characterization of baseline microbial associations in a range of environments (wild, field nursery, land-based); 3) Evaluative studies to optimize a wide range of ‘how’ questions, including but not limited to attachment methods, scale/density of outplants, orientation, 4) improve collaborative knowledge sharing (perhaps formal meta-analysis) of outplanting methods and results
- Engage regulators in these recommendations as permitting is currently a primary constraint on outplant design.

There are a myriad of factors to consider in planning and implementing a successful coral out-plant. Generally, these involve the nature/condition of the outplants themselves, and the nature/condition/configuration of the site where they will be placed. The next two sections are organized accordingly.

Health/Condition of the Outplants

It may be argued that these considerations vary somewhat between *in-situ* and *ex-situ* culture conditions (or a continuum thereof). Generally, any cultured coral is subject to ‘unnatural’ conditions which, in turn, may lead to alterations in their health status, and this alteration may be considered a matter of degree across different culture conditions. First, obtaining ‘healthy’ and appropriate (e.g., appropriate ecotypes for the culture and anticipated outplanting conditions) stock material for culture is always a first step to both cost-effective and successful culture. Second, good husbandry practices are crucial in maintaining and producing healthy and appropriate cultured stock. The former aspect is treated largely in the two culturing practices (and to some extent the genetics) working groups. This section will focus on the husbandry and determination of healthy material for outplanting.

Given the depth of our ignorance concerning appropriate characterization of the true health status of corals, guidelines for husbandry and condition criteria are currently largely restricted to common-sense approaches. Namely, culture facilities (either land-based or field based) should strive to provide environmental conditions (water quality, light, temp, water motion, microbial cycling) that are matched as closely as possible to the intended outplanting site(s). Also, particularly in *ex-situ* culture conditions, basic bio-security protocols must be strictly applied (e.g., prevent exposure or water mixing with exotic species, strict assurance against toxic exposures). Biosecurity protocols are an appropriate requirement for regulation/permitting of outplants. Outplants should present at least a visual suggestion of health (i.e., robust coloration and lack of active tissue loss or lesions). However, it should be emphasized that **we currently lack appropriate tools or diagnostics to determine the true health state of a coral.** Husbandry requirements and visual assessments are appropriate to reduce the risks that might accrue to outplanting unhealthy corals, but they should not be construed as constituting a ‘health certificate’ or assurance of health or success of the out-plants. Therefore there is a need to develop guidance on what are the ‘best husbandry practices’ leading to successful outplanting in general and then in more specific language based on a given location or habitat type. Similar guidance needs to be developed for biosecurity and biocontainment practices for this application. This includes rapid removal and monitoring of moribund corals within nurseries. This provides an important opportunity for future work.

This point emphasizes a primary point of discussion and of the health working group’s deliberation: the immense need for specific, sensitive and cost-effective diagnostics that are amenable to performing in field conditions to accurately determine health status of the entire coral holobiont (and microbes are likely a substantial component of this healthy condition). Steps to develop such diagnostics include better characterization of baseline ‘health’ conditions in corals across a range of wild habitat types, nursery, and *ex-situ* conditions, and across time/season.

A range of technological approaches may prove useful in this quest and some candidate methods that were suggested include:

- Development of ‘indicator water column bacterial assemblages’ that would indicate healthy corals while reducing need to sample the corals directly
- Bio-optical properties of corals that could characterize healthy coral/zoox conditions (Hochberg et al, 2006) as well as auto-fluorescence profiles from the cnidarian host which is difficult because it variable
- Genomic tools such as stress-gene microarrays (Edge et al), the use of other reef proxy organisms [e.g., sponges, whose microbial ecology is relatively better characterized]
- Adaptation of innovative technologies from the broader medical field e.g., hyperspectral imaging in diagnostic medicine

For example, one potentially useful diagnostic method to obtain baseline microbial loads would employ “Phylochip-like” arrays, populated with specific marine microbial 16S rRNA signature sequences, including both known coral symbionts and pathogens (Yergeau et al, 2008). This method could be applied to subsamples of different *ex situ* or *in situ* *Acropora* individuals, or to sea water and aquaria water samples that were suggested as possible proxies. Other sensitive molecular techniques such as fluorescent *in situ* hybridization (FISH), real-time qPCR, and metagenomics can also be effectively applied for more detailed profiling of microbial communities (Cassler et al, 2008; Negandhi et al, submitted, Vega-Thurber, 2009). Although some of these approaches and methods are highly technical and costly, support by a central agency which has broad authority could help in their implementation.

We need to strive for applied technologies that can be used on-location. Using phylochips or related technologies should not be the target diagnostic but rather the research/development phase to get to an *in situ* application.

Site Selection

General considerations for choosing appropriate out-plant sites include:

- Bottom type (minimal loose material)
- Water quality
- Predator abundance
- Wave exposure
- Accessibility
- Protected area status
- General matching of environmental conditions to those of the culture situation and/or to the original stock collection site (both geographically and habitat-wise)
- Potential ‘downstream’ habitat that could benefit from larval production
- ‘Value’ or visibility of the site with regard to tourists or other stakeholders (also see Stewart et al, 2008)

Permitting is also often a formidable constraint in siting outplants and may preclude other considerations. Specific project goals may dictate prioritizing some site characteristics over others. A small scale pilot out-plant is an ideal means to determine performance of out-plants (including potential differences among genotypes) at a range of environmental conditions across several sites. While an ‘optimal’ site (e.g., based on pilot results) is a logical target for initial outplanting efforts, the broader interest in maintaining genotypic diversity at metapopulation scales suggest that out-plants should also be carried out at ‘non-optimal’ sites. Evidence of previous presence of the species (e.g., from historical maps if available, or presence of rubble) is a positive sign.

When trying to ‘re-stock’ an area, it is critical to investigate what stressor(s) were involved in the initial demise of the previous corals and determine if those threats have been abated at a particular location, otherwise the effort seems at best a gamble, at worst in vain. To achieve a level of confidence that the site has been ‘cleaned-up’ will likely require not only water quality but in many cases contaminant testing and other more analytical measures.

Outplant Implementation

How many out-plants, of what size, and in what configuration are also daunting considerations and, while many practitioners have experience and knowledge regarding good practices, optimizing these aspects remains an important research focus. Recent experimental evidence suggests that the ‘coral neighborhood’ (i.e., density and identity of out-plant neighbors) of out-plants can affect colonization and impact by corallivorous snails. Keep in mind that *Acropora* spp. corals will grow so do not place them too close together (or to *in situ* corals).

There was more disagreement on the appropriateness of post-outplant maintenance or ‘gardening’ activities. On one hand, this seems to violate the stated goal to establish self-sustaining patches of coral. However, predator/competitor removal can be very effective in enhancing growth/establishment of transplants and can be a very constructive means to engage local stakeholders or volunteers, at least for a period of time after outplanting (i.e. not in-perpetuity).

Research Priorities

1. Develop tractable diagnostics for the health status of corals which accurately predict success of out-plants and minimize risks posed to the receiving ecosystem.
2. Characterization of the normal temporal and spatial dynamics of coral-associated microbial communities. This characterization must move from only characterizing which bacteria are present to what functions are being performed a healthy condition and is this functionality maintained with outplanting (meaning it may be more important to know that certain ‘healthy’ functions are occurring than to know who’s in the community] Experimental transplant studies to determine whether or not the microbial consortium can acclimate to out-planted conditions. These results will provide basis to better understand degree (if any) of increased risk relative to closed, land-based culture, and/or between short-term holding vs. long-term culture.
3. Optimize configurations and densities of transplants, fragment size and attachment methods to ensure success (considering genotypic and habitat characteristics) across habitats and geography.

4. A region-wide approach to collecting information, lessons learned, challenges, success (perhaps a meta-analysis) of outplanting activities should be conducted. This is necessary to form a collaborative effort in teasing out the recurring issues. An online, secure platform could facilitate sharing information.
5. Novel asexual propagation methods, such as tissue ex-plantation, should be explored to more fully understand conditions that optimize growth and development and, in so doing, also help identify factors in field conditions that may impede establishment and growth of out-planted corals. Associated with tissue micropropagation are cryopreservation methods used in tissue and organ cryopreservation that may be adaptable allowing whole tissue preservation and banking.

Other research priorities (no priority order) include the following:

- Monitoring for spawning and larval abundance near out-planted populations and restoration sites should be researched. Permanent quadrats can be used. A map of larval abundance could then be developed and visually determine where spawn masses are moving from place to place. It will give an idea of natural seeding potential.
- Improved understanding of the importance of intact trophic structure and predator life-history characteristics in determining outplant success. This could potentially validate the benefits of marine reserves in outplanting activities.
- Understanding disease etiology and dynamics from (pathogenesis, to pathogen-host interactions, toxins, etc.) and potential interventions in order to minimize disease impacts
- Integration of eco-system wide variables (correlations between physical characteristics interaction with the transplants and the effects of that) should be considered for research. For example environmental monitoring devices such as the “Kilroy” sensor invented and distributed by the ocean conservancy group, ORCA (<http://www.teamorca.org/cfiles/about.cfm>; http://www.eurekalert.org/pub_releases/2009-02/orc-otf021909.php) could be placed at both nursery and candidate outplanting sites prior to seeding to measure important dynamic, physical oceanic parameters, and obtain diagnostic baseline data for coral outplanting survival.
- Challenge experiments to identify resistant clones for the purpose of selection to produce resistant out-plants. However, there are many variables to consider: evolution of sites, maintaining genetic diversity, etc., and such physiological experiments are also very difficult to carry out successfully.

A caution about selection for resistance is that consideration should be given that resistance to one threat may subject the organism to susceptibility to another, so care must be given to these practices. Especially if the outplants out compete *ex-situ* existing resilient colonies or populations.

References:

Cassler M, Peterson CL, Ledger A, Pomponi SA, Wright AE, Winegar R, McCarthy PJ, Lopez JV. 2008. Use of real-time qPCR to quantify members of the uncultivable heterotrophic bacterial community in a deep sea marine sponge, *Vetulina* sp. *Microb Ecol.* 55(3):384-94.

- Edge SE, Morgan MB, Gleason DF, Snell TW. 2005 Development of a coral cDNA array to examine gene expression profiles in *Montastraea faveolata* exposed to environmental stress. *Mar Pollut Bull.* 51(5-7):507-23.
- Hochberg, EJ., Apprill, AM., Atkinson, MJ., Bidigare, RR. 2006 Bio-optical modeling of photosynthetic pigments in corals. *Coral Reefs* 25: 99–109
- Negandhi, K., Blackwelder, P., Ereskovsky, A.V., Lopez, J.V. Florida Reef Sponges Harbor Coral Disease-Associated Bacteria. *Symbiosis Journal*. Submitted
- Stewart JR, Gast RJ, Fujioka RS, Solo-Gabriele HM, Meschke JS, Amaral-Zettler LA, Del Castillo E, Polz MF, Collier TK, Strom MS, Sinigalliano CD, Moeller PD, Holland AF. 2008 The coastal environment and human health: microbial indicators, pathogens, sentinels and reservoirs. *Environ Health.* 7;7
- Vega Thurber R, Willner-Hall D, Rodriguez-Mueller B, Desnues C, Edwards RA, Angly F, Dinsdale E, Kelly L, Rohwer F. 2009. Metagenomic analysis of stressed coral holobionts. *Environ Microbiol.* 2009 Aug;11(8):2148-63.
- Yergeau E, Schoondermark-Stolk SA, Brodie EL, Déjean S, DeSantis TZ, Gonçalves O, Piceno YM, Andersen GL, Kowalchuk GA. 2008. Environmental microarray analyses of Antarctic soil microbial communities. *ISME J.* 3(3):340-51.

Genetic Considerations Related to Outplanting

Summary:

- Genetic concerns relate to 1) potential loss of genetic diversity within the wild population and its consequences; 2) appropriate genetic design of restocking in order to maximize the likelihood of meeting restoration goals under given investments of finances and effort
- Consensus for the greatest concerns related to genetic status of the species included potential for outbreeding depression in mating of disparate genotypes, emerging evidence that genetic drift has already affected *A.cervicornis* (and possibly *A.palmata*) populations, and the challenge of assessing (let alone maintaining) genetic diversity among other holobiont components (zooxanthellae and microbial associates). Inbreeding depression was not considered a current concern (though it could be if poorly implemented restocking was undertaken).
- In implementing propagation and restocking activities, the goal should be to incorporate a hierarchy of genotypic diversity include 1. East/west population break (for *A.palmata*), 2.Regions within east/west, 3.Ecotones within regions, 4.Targeting both ‘good’ and ‘bad’ reefs or targeting at- risk populations.
- Outplants must be designed with appropriate density and genotypic diversity to maximize recovery goals including increased larval production. Experts suggested colony distances of 0.5m to 10m and 1m to 10m for *A. cervicornis* and *A. palmata*, respectively, for outplants. However, this aspect requires experimental evaluation over a medium term (5-10 yrs).
- An additional research need is the investigation of among-genet reproductive compatibilities with the goal of developing tractable genetic screening tests in order to allocate genets in outplant groups to maximize reproductive output.

Discussion:

The genetics working group identified two main concerns when considering the genetic implications of Caribbean acroporid populations:

1. The loss of genetic diversity in naturally occurring populations
2. The potential for failure to restore sexually reproducing acroporid populations during restocking efforts due to inappropriate genetic design

The genetic working group initially considered if enhancing genotypic diversity or artificially selecting for genets that are resistant to specific afflictions (i.e. disease, predation, bleaching, etc.) is appropriate. The group decided to focus our efforts on maintaining genotypic diversity so that natural selection can act on populations without human intervention of artificial selection. If acroporids continue to decline and genetic diversity is lost, the enhancement of genotypic diversity and artificial selection may eventually be necessary.

Current genetic diversity is high throughout the Caribbean in both *A. palmata* and *A. cervicornis*; however, based on individuals from the Florida Keys and Mexico populations, it appears that a bottleneck has occurred in *A. cervicornis*. Inbreeding, outbreeding, genetic drift, and recognizing the diversity of symbionts were all discussed as potential concerns relating to loss of genetic

diversity. **Inbreeding** is not currently considered a threat to wild populations. If outplanting of lab-reared siblings from the same parents to the same reef would repeatedly occur, inbreeding depression could have negative consequences in acroporids. It was suggested that genotyping all fragments used in restoration efforts would help ensure diversity. In addition, a studbook to track asexual fragments and sexually reared larvae would assist in maintaining diversity at restoration sites and at aquaria.

Outbreeding depression was broken down into hybridization between *A. palmata* and *A. cervicornis* and mating between two distantly related individuals within the same species (e.g., crossing *A. cervicornis* individuals from the Mexico population with *A. cervicornis* in the Florida Keys). Throughout the Caribbean, hybridization occurs between *A. palmata* and *A. cervicornis* and, in some locations, hybrids outnumber one or both of the parental species. In order for outbreeding depression to occur, the hybrid would have to have reduced fitness. Hybrids are fertile when crossed with each other and when crossed with *A. cervicornis*, and they are less susceptible or equally susceptible to the afflictions (i.e. disease, predation, bleaching, parasitism) that plague the parental species and has led to their decline.

Hybridization can also provide increased genetic diversity in depauperate populations. It is unlikely that outbreeding depression because of hybridization is occurring in this system. Because there are no barriers to hybridization, introgression (i.e. gene flow) of genes from *A. palmata* into *A. cervicornis* may lead to the eventual swamping of *A. cervicornis*' genome on an evolutionary timescale. Hybridization appears to relate directly to the density of the parental species; therefore, the likelihood of this occurring may hinge on the ability of these species to avoid further loss from current biological and environmental stressors. There is increasing evidence that local adaptation occurs in acroporid populations, and thus outbreeding depression may result when (very) distantly related individuals mate. In order to avoid this, moving individuals or mixing gametes of individuals from distant populations should be avoided. Instead, individuals should be transplanted to physically close and environmentally matched sites (i.e. inshore to inshore, offshore to offshore).

There is preliminary evidence that **genetic drift** has occurred in *A. cervicornis* and it is likely that it will or has already occurred in *A. palmata* as well. The effects of genetic drift are magnified when population size is small; therefore, the best buffer to ensure genetic drift does not decrease the genetic diversity is to increase the population size. Genetic drift may be problematic if/when captive bred corals become sexually reproductive and therefore genetic drift may need to be addressed in order to properly manage captive populations.

Managing corals presents unique challenges, in that we are not only managing the coral animal itself but the holobiont which includes the coral host, the microbial community, and the symbiotic dinoflagellate, zooxanthellae. Because the **holobiont is likely locally adapted** to a specific habitat, moving corals may decrease their likelihood of survival. We are only beginning to understand the diversity of zooxanthellae that exists in natural populations. Currently, the best practice is to be aware of the ecotone in which corals live, and to try to maintain corals in the ecotone for which they are adapted. Future considerations include a workshop be held to understand and manage holobiont diversity, and perhaps establish captive bred clonal lines of zooxanthellae to act as an insurance policy for maintaining genetic diversity.

The second major concern discussed in the genetics working group is that much time and money will be expended, but restocking efforts may fail to meet recovery goals, defined by this working group as follows:

1. Save the species from extinction
2. Maximize reproductive output
3. Establish ecosystem services
4. Increase abundance and diversity

Overall, sexually reproducing thickets of *A. palmata* and *A. cervicornis* over a large proportion of their existing geographic range will constitute success.

In order for sexual reproduction to be maximized, the design of the restocking efforts will need to be carefully considered. Potential ways to create a population that can successfully fertilize will depend upon the density and genotypic diversity of the colonies. If coral colonies are transplanted too close to each other some genets may be outcompeted and genotypic diversity will be lost. If coral colonies are placed too far apart fertilization may be reduced.

The proposed minimum and maximum distances colonies should be placed is 0.5m to 10m and 1m to 10m for *A. cervicornis* and *A. palmata* respectively. This is an estimate based on expert opinion, but should be tested in a controlled experiment. Genotypic diversity will also dictate successful fertilization. Compatibility is highly variable between individuals within a species; therefore genotypic diversity is needed to increase the probability of fertilization success. Though Caribbean Acroporids are hermaphroditic, self-fertilization is very low for *A. palmata*, but average selfing rates in *A. cervicornis* are much higher (20%). Although self fertilized larvae in both species are viable through settlement, it is not clear that any of the self fertilized larvae survive to adulthood.

Capturing species-level genotypic diversity within restocking efforts should be represented in a hierarchical fashion:

1. East/west population break
2. Regions within east/west,
3. Ecozones within regions
4. Targeting good/bad reefs equally or target at risk populations.

To ensure genotypic diversity, replicate outplant arrays of mixed genotypes within a population should be established.

One area of research is to investigate reproductive compatibility among genets with the idea of developing diagnostics for compatibility such as DNA or protein markers for developing more field-friendly assays for screening colonies for compatibility.

Considerations in Forming a Consortium

Need

As coral reef scientists, we are facing perhaps the most daunting task thus far: saving key coral species from possible extinction. Although numerous research projects, meetings, workshops, management strategies, and restoration efforts have been undertaken over the past few decades, evidence of coral reef (and Atlantic acroporid population) decline continues to mount. This current challenge may define our ability to address all others: if we cannot prevent the loss of viable acroporid populations from the Western Atlantic Province, how can we have hope for saving reefs in general? As more coral species face a similar crisis, what we learn from this effort will have broader applications.

This working group was tasked to examine whether efforts to address the plight of Atlantic acroporid corals should be coordinated by an international consortium of academic, governmental, conservation, and private organizations. With NOAA's mandate to produce a recovery plan for the Atlantic acroporids within U.S. waters (<10% of the species range), one of the prime reasons for an international consortium would be to insure that coordinated recovery and conservation plans are applied throughout the wider region where these species exist. If finding in favor of such a consortium, this working group's tasks also include providing a mission, primary goals, and recommendation for further action.

The consortium working group recommends the formation of a consortium as key to the international coordination of the many resources that will be required to rescue declining coral populations from localized extinction and, if possible, to eventually return them to their preeminent position on reefs. However, the ultimate goal of such a consortium is to put itself out of business through insuring sustainable reef coral populations, and thus delisting of corals from national and international lists of threatened and endangered species. As a working name, the "International Endangered Coral Consortium (IECC)" is suggested.

Scope

In considering the following mission statement, goals and recommendations for further action, the working group envisioned the scope of the IECC to be focused, in the near term, on the Atlantic acroporids: *Acropora cervicornis*, *A. palmata*, and *A. prolifera*. Some of the goals reflect this initial limitation. However, the work by this group should provide information, resources, and collaborative relationships that allow it to address additional species and geographically expand to include other reef coral biogeographical provinces as deemed beneficial to recovering coral populations in general.

Mission Statement

The IECC will develop and facilitate the implementation of international species recovery and conservation plans for threatened and endangered reef corals.

Primary Objectives

1. Develop an International Atlantic *Acropora* Recovery and Conservation Plan, generally based on the final NOAA *Acropora* Recovery Plan. The international plan will include modifications to NOAA's plan to reflect the entire biogeographic range of Atlantic acroporids and accommodate local needs and conditions.
2. Facilitate dialog and collaboration amongst consortium members and foster collaborative partnerships between consortium and additional stakeholders (i.e. academia, governments, private sector, etc.) to engage in research and implementation projects, when and where appropriate
3. Secure funding for consortium activities to formulate and carry out international restoration plans including helping to identify and secure funding for constituent projects
4. Coordinate, and develop as necessary, resources for *in* and *ex situ* genetic repositories/nurseries of reef corals including proper quality control measures and maintenance of genotypic records (studbook).
5. Coordinate, and develop as necessary, resources for *in situ* restoration of threatened and endangered reef coral populations including ongoing monitoring efforts
6. Help coordinate, and develop as necessary, resources for the monitoring of wild populations of threatened and endangered corals
7. Produce a manual of "best practices" for methods recommended within coral recovery plans
8. Provide educational resources and training opportunities for scientists, students, resource managers and volunteers
9. Contribute to public outreach efforts to promote conservation of corals, reefs and general conservation through diverse media, presentations and public venues such as aquaria and zoos

Recommendations for Further Action

1. Form IECC Steering Committee. It is suggested that the following constituencies each have representation: academia, NGO conservation/research organizations, public aquaria/zoos, governmental marine resource agencies, IUCN/SSC's Coral Specialist Group, other intergovernmental organizations (i.e. World Bank, CARICOM, etc.), dive industry, and marine collectors. It is also suggested that members represent a range of expertise germane to the recovery and conservation of acroporids.
2. Selection of Steering Committee Chairperson and any other officers as deemed necessary
3. Finalization of the name, mission, goals, and by-laws by the Steering Committee
4. Solicit member institutions with formal letters of interest and designated representatives from all interested stakeholders
5. Seek and obtain organizational funding and in-kind services
6. Recruit IECC core staff as necessary

7. Establish interactive website with public interface and collaborative resource pages for member institutions
8. Establish working groups
9. Seek funding for facilities and projects based on recommendations of working groups
10. Help coordinate projects related to coral Recovery and Conservation Plans, including maintenance of a database tracking project activities and results

Suggested Consortium New Working Groups






1. Recovery Plan – To develop International Atlantic *Acropora* Recovery and Conservation Plan based on NOAA *Acropora* recovery Plan. (Note: If recovery and conservation plans are to be developed for additional species or related species groups, it is recommended that a working group be established for each with appropriate expertise.)
2. *In situ* Repositories/Nurseries – Assemble information, methods and best practices for *in situ* coral nurseries
3. *Ex situ* Repositories – Assemble information, methods and best practices for *ex situ* genetic repositories. This may include subgroups for genetic material, tissues and gametes, closed system culture, and open system culture.
4. Sexual Reproduction – Assemble information, methods and best practices for collecting gametes, fertilization and settlement. SCORE would be the logical group to serve this function here.
5. Population Replacement & Enhancement – Assemble information, methods, and best practices for colony out-planting
6. Wild Population Monitoring and Interventions – Assemble best practices for mapping, monitoring, and care of wild coral populations to increase population health. Also, develop research methods for testing the efficacy of “gardening” (weeding, predator removal, etc) of wild populations.
7. Fundraising and Partnerships – Identify funding opportunities and secure resources to support Consortium mission and activities. Identify strategic partnerships to support Consortium mission and activities.
8. Communications and Outreach – Promote Consortium mission, expertise, and significance through on-line resources (i.e. web page, wiki sites, list server), printed materials, and public outreach efforts
9. Training – Oversee development and publication of Methods Manual and other training materials. Conduct training workshops for students, resource managers and volunteers.
10. Permitting – Assemble information for Consortium members regarding local and CITES permit requirements for all countries in which Consortium activities are likely to occur
11. Population Status – Coordinate efforts and information regarding ongoing status of *Acropora* populations across the Western Atlantic Province. The work of this group will be key to continuing implementation of the Recovery and Conservation Plan and (hopefully) eventual delisting.

Appendix 1: Beyond the Workshop












The seeds of future work are mentioned throughout this document. The discussions repeatedly came back to the need to produce written accessible manuals that capture the experience of field scientist, nursery managers, aquarist, regulators, and other practitioners. The need to form a consortium to continue this work was identified as well. Highlighting the link to climate change was identified as a means to seek much needed resources to continue this work. Future grants must be sought for this work. This workshop advances the compiling of many years of hard work and research already done, and provides a foundation to bring together many more practitioners and stakeholders. As manuals are produced they will provide feedback for and contribute to the implementation of an Acropora recovery plan soon to be revealed by NOAA. The individual work and manuals, meetings, and electronic technologies are creating a synergy that will advance the movement to save corals and our planet.



Appendix 2: Participant List

The workshop participants are listed below as registered on the Acropora Coral Conservation Restoration wiki site used to coordinate this project through the MAX system and run by the U.S. Office of Management and Budget.

 BAUMS, ILIANA	ibb3@psu.edu	814-867-0491
 BORNEMAN, ERIC	eborneman@uh.edu	713-589-9869
 BOWDENKERBY, AUSTIN	bowdenkerby@yahoo.com	679-938-6437
 BRUCKNER, ANDY	andy.bruckner@noaa.gov	301-577-1288
 BRITTSAN, MIKE	mike.brittsan@columbuszoo.org	614-724-3580
 BYERS, ONNIE	onnie@cbsg.org	952-997-9801
 CARL, MITCH	mitchc@omahazoo.com	402-557-6933
 CARNE, LISA	lisas@btl.net	
 FOGARTY, NIKKI	fogarty@bio.fsu.edu	305-923-0683
 GILLIAM, DAVID	gilliam@nova.edu	954 232-1035
 GRIFFIN, SEAN	sean.griffin@noaa.gov	787-667-7750
 HAGEDORN, MARY	hagedormm@si.edu	808-520-1368

 HENLEY, EMMETT M	henleym@si.edu	202-633-3875
 HERNANDEZ, EDWIN	coral_giac@yahoo.com	787-764-0000
 HICKS, CHRISTINE	chicks@counterpart.org	703-236-3285
 HOFFMAN, BILL	hoffman@si.edu	
 HOLECEK, ALEX	alex.holecek@gmail.com	340-244-8252
 HUTCHINS, AARON	ahutchins@tnc.org	340-718-5575
 JOHNSON, MEAGHAN	meaghan_johnson@tnc.org	305-745-8402
 KELLER, BRIAN	brian.keller@noaa.gov	727-553-1201
 LATERVEER, MICHAEL	m.laterveer@rotterdamzoo.nl	202-633-4292
 LILYESTROM, CRAIG	craig_lilyestrom@drna.gobierno.pr	787-999-2200
 LIRMAN, DIEGO	dhirman@rsmas.miami.edu	305-421-4168
 LOPEZ, JOSE	joslo@nova.edu	954-262-3665
 MCFIELD, MELANIE	mmcfield@gmail.com	501-670-3953
 MICKELBERG, JENNIFER	mickelbergj@si.edu	202-633-4203

 MILLER, MARGARET	margaret.w.miller@noaa.gov	305-361-4561
 MOORE, TOM	tom.moore@noaa.gov	727 551-5716
 MOORE, JENNIFER A	jennifer.moore@noaa.gov	727-551-5797
 MUELLER, ERICH	evmresearch@gmail.com	231-645-4556
 NEDIMYER, KEN	sealife1@bellsouth.net	305-942-3250
 PETERSEN, DIRK	d.petersen@rotterdamzoo.nl	202-633-4292
 PETERS, ALAN M	petersam@si.edu	202-633-4292
 RINGELSPAUGH, BUTCH	bringelspaugh@pieraquarium.org	727-421-6394
 RITCHIE, KIM	ritchie@mote.org	941-388-4441
 ROSS, ANDREW	ross.andrew@mac.com	876-363-8850
 SCHICK, MARK	mschick@sheddaquarium.org	312-692-3189
 SHARP, BILL	bill.sharp@myfwc.com	305-289-2330
 SZMANT, ALINA	szmanta@uncw.edu	910-962-2362
 WILLIAMS, RAPHAEL	williams@si.edu	772-462-0988

 WOODLEY, CHERYL M	cheryl.woodley@noaa.gov	843-762-8862
 YOUNG LAHIFF, CHELSEY	cyoung@rsmas.miami.edu	954-263-1611



Appendix 3: Acknowledgements

The Acropora Coral Conservation and Restoration Workshop was a cross-institutional and multi-national effort to help save Acropora corals. This November 12-13, 2009 workshop and resulting report has been made possible by the work and support of NOAA, Smithsonian Institution's National Zoo, and Counterpart International. A great deal of thanks and gratitude has been extended to the supporting Smithsonian's staff and volunteers for executing the workshop. A thank you has also been sent to the US Office of Management and Budget for the use of the MAX community computer network. The true credit and an appreciation for giving a significant amount of time, sharing valuable experience, and putting forth excellent effort at the workshop goes to each and every participant.