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CARIBBEAN CORAL REEFS IN PERIL AND THE ADVANCEMENT OF CONSERVATION: *EX SITU* SETTLEMENT AND REARING OF *ACROPORA PALMATA* AS A MECHANISM FOR CORAL REEF RESTORATION IN THE CARIBBEAN

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(Andrew Hinrichs, St. Olaf graduate of 2010, provided text for this summary of the keynote address delivered by Eric Borneman: noted author, aquarist and coral restoration advocate. Figures, where noted were generously provided by E. Borneman.)

ABSTRACT

Throughout the Caribbean, coral reefs have undergone dramatic changes as a result of pollution, disease, and global climate change. The consequences of human activity have led to the degradation of coral reefs to an extent that their geological, physical and biological foundations have been compromised. Diverse efforts have been implemented in the Caribbean to protect and restore the ecological benefits and economic resources that coral reefs provide. Conservation strategies have employed the use of offshore nurseries, creation of artificial reefs, and storm generated coral fragments for reef rehabilitation. The SECORE (SExual COral REproduction) project is committed to research focusing on coral restoration and the endangered Elkhorn coral (*Acropora palmata*) in Puerto Rico. An international initiative of scientists from public aquariums and research organizations dedicated to Caribbean coral reef conservation, SECORE has focused on coral reef restoration through in-field collection of naturally spawning A. palmata gametes, *ex situ* settlement of larvae and rearing to juvenile colonies. Following demonstration of feasibility, SECORE will explore opportunities for transplantation of colonies to Caribbean reef sites. SECORE has cultured over 2.5 million A. palmata larvae with viable transportation of 900,000 colonies with a 5-36% settlement rate. Survival at three months has been between 5-30% with 2,000 living colonies to date. Previous efforts have produced as few as two colonies. Currently, public aquariums in five countries are studying and displaying these corals raised from larvae. Future SECORE efforts will focus on the development of a seaside cultivation facility and transplantation of *ex situ* reared coral onto degraded reefs. SECORE strategies for *ex situ* coral propagation are expected to lead to greater understanding of factors that contribute to future success in preserving one of the earth's most diverse ecosystems, the coral reef.

Keywords: Coral reefs; Caribbean; SECORE; *Acropora palmata*; spawning; settlement; recruitment; *ex situ*

INTRODUCTION

Coral reefs worldwide are in serious decline because of pollution (McCulloch et al. 2003), disease (Harvell et al. 2002), and global climate change (Hughes et al. 2003). The deterioration of the world's coral reefs endangers global biodiversity, ecosystem longevity, and people that rely on the oceans for subsistence. In particular, corals in the Caribbean have encountered both global and local stressors. Local stressors that have impacted scleractinian corals in the Caribbean include predation (Weil 2003), disease (Precht et al. 2002) and sedimentation (Nystrom et al. 2000). Specifically, *Acropora palmata* and *A. cervicornis*, have shown reductions in population by 80-100% throughout their range in the Caribbean since the 1980's (See Figure 1 and 2).

Figure 1 and 2. Above, historical photograph of healthy A. palmata. Below, current stand of A. palmata. Photographs by Eric Borneman.

Acroporid corals play a major role in Caribbean reef communities by providing geological, physical, and biological foundations for the development of shallow reef communities. These corals provide essential food, refuge, and recruitment habitat for fish, turtles, lobsters, crabs, echinoids and gastropods (Bruckner 2002). In addition, Acroporid growth rates and structural complexity are unmatched by any other Caribbean coral species. There is no indication that any other Caribbean coral species can replace the important role that Acroporids play within reef communities of the region. Many different approaches have been implemented in the Caribbean in order to manage and protect the ecological roles and economic resources that coral reefs provide. In areas where reefs have deteriorated greatly, efforts have focused on developing restoration techniques, including offshore nurseries, artificial reefs, and the use of storm-generated coral fragments as an aid to reef rehabilitation (Garrison and Ward 2008). Although these strategies have resulted in new methods and techniques, many are labor intensive and need constant maintenance. Other scientific gaps include unanswered questions concerning how water quality is affecting the reduction of Acroporid corals. A significant lack of information persists pertaining to reproductive cycles and genetic diversity, especially for *A. cervicornis*.

Despite ongoing conservation efforts, Acropora corals do not appear to be recovering and there is reason to believe larval recruitment (settling of coral planula larvae on coral reef substrate and metamorphosing into juvenile colonies) is not occurring at a rate that allows for recovery of reefs. Degraded reefs exhibit declining rates of coral recruitment, related to reduced adult fecundity, lower settlement rates, and higher rates of early mortality (Bellwood et al. 2004). Therefore, unless regenerative capacities can be understood, increased, and actively managed, the long-term survival of *A. palmata* in the Caribbean will remain threatened.

CORAL REEF CONSERVATION AND SECORE

SECORE (SExual COral REproduction) is one project that is committed to research focusing on coral restoration and the endangered coral *A. palmata* in Puerto Rico. Initially developed by Dr. Dirk Petersen and Michael Laterveer (Rotterdam Zoo, Netherlands), SECORE is an international initiative of public aquariums and research organizations dedicated to Caribbean coral reef conservation. SECORE utilizes coral development and life cycle knowledge as the basis for development of restoration techniques. Other related projects include studies on genetic variation of *A. palmata* gametes, cryopreservation of *A. palmata* sperm gametes, and public awareness lectures.

Corals have evolved a wide range of reproductive strategies to persist over time. Besides asexual methods of reproduction, corals are capable of wide variability in sexual reproduction. A majority of coral species are hermaphrodites (polyps are both male and female), while the remaining third of all species of coral in the Caribbean are gonochoristic (an individual coral produces either sperm or egg). *A. palmata* is a simultaneous hermaphroditic breeding coral. Following the broadcast spawning or release of *A. palmata* gametes, sperm and egg bundles are exposed to surge and wave action that induces the bundles to break apart and allow for cross fertilization or union between sperm and egg of neighboring coral colonies.

After fertilization, the egg becomes a planula larvae—a heavily ciliated, microscopic, and elongated structure—and may remain in a planktonic phase for weeks until finding a potentialsubstrate for settlement. A complex searching behavior facilitates the determination of a suitable substrate. Once a substrate has been chosen, the larva settles and metamorphoses into a coral polyp no bigger than one millimeter. The period following settlement is a vulnerable timeframe for the developing coral polyp. The coral is exposed to macroalgae competition, sedimentation, and corallivores. Should a coral be overgrown by algae or covered by sediment, the coral will most likely die. The evolutionary strategy of producing large quantities of gametes is thought to help corals ensure the success of sufficient propagules so that the species will survive.

Following settlement, *A. palmata* larva will acquire endosymbiotic dinoflagellates called zooxanthellae algae. The zooxanthellae are important to the coral because they provide energy-rich organic compounds through photosynthesis. In return, the coral provides a home for the algae through the excretion of calcium carbonate to form a skeletal structure. The by-products of cellular respiration from the coral also provide nitrogenous wastes and CO_2 for the zooxanthellae algae (Pechenik 2010).

SECORE FIELD WORK

In 2005, the 1st SECORE workshop successfully established on-site training programs for aquarium biologists in breeding techniques for various Caribbean corals, identification of larvae and primary polyps. Zoo and aquarium facilities were also selected for establishing breeding and *ex situ* propagation centers. In 2006, at the 2nd SECORE workshop, SECORE narrowed its efforts by primarily focusing on the conservation of *A. palmata*. SECORE established study sites for *A. palmata* populations in the Tres Palmas Marine Reserve as well as Bajo Gallardo, Puerto Rico.

Despite concerns for the predictability of *A. palmata* spawning in Puerto Rico, SECORE was able to calculate possible spawning events based on lunar cycles and tide changes. Release of gamete bundles (small capsules with separated compartments of sperm and egg) occurred between 9:30 PM and 10:30 PM, three to four hours after the August full moon during the 2006 2nd SECORE workshop. During predicted spawning events, divers would work in teams of two. One diver using a hand-made, larvae net in the shape of a funnel with a 500mL nalgene attached to the end for collection of gametes. The other diver was responsible for helping with lighting the diving area and changing nalgene bottles when filled with gametes. Following the spawning event, gametes where transferred to the beach for fertilization. Two hours after fertilization, fertilized eggs were transferred to large containers for development. Water changes were completed on the larvae containers with filter, natural seawater. For a complete review of SECORE collection, fertilization, and culturing methods, see (Petersen 2008).

When not conducting field experiments or larvae culturing, scientists and aquarium specialists continued classroom education sessions pertaining to Caribbean coral reef natural history, coral reproduction, and larval settlement techniques. Large group sessions also included laboratory training for field collection of coral gametes, fertilization techniques, and seaside larvae husbandry. During the 2006 season, SECORE successfully reared one million larvae in the field and transported the developing larvae to various public aquaria in the United States. After larvae were settled onto specially designed ceramic tiles (See Figure 3) at public aquaria, primary polyps were transferred to grow-out systems for long-term development. After one year, 821 individual *A. palmata* colonies were thriving in captive aquaria systems. This achievement by SECORE is the most successful rearing of *A. palmata* using *ex situ* development strategies (Pennisi 2007) (See Figures 4, 5, and 6).

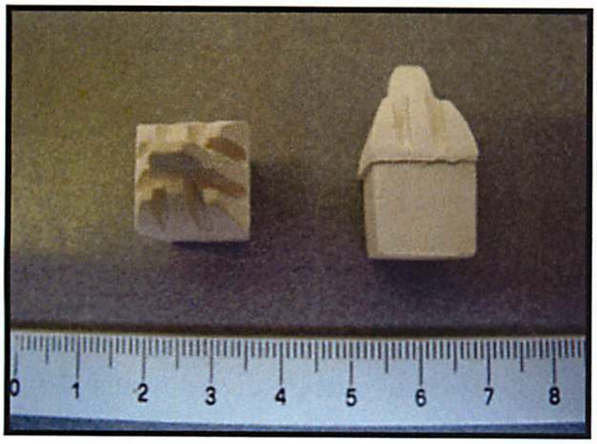

Figure 3. Ceramic settlement tiles for planula larvae. Photograph by SECORE.

Figure 4. A. palmata colony at three months. Photograph by Mitch Carl.

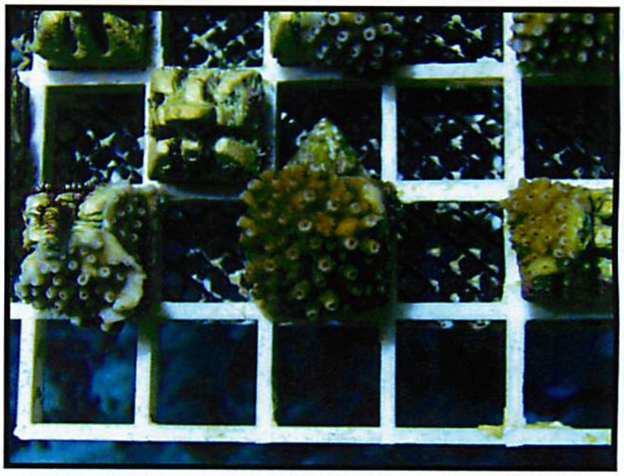

Figure 5. A. palmata colony at one year. Photograph by Andrew Hinrichs.

Figure 6. A. palmata colony at three years. Photograph by Andrew Hinrichs.

Challenges that occurred during the 2nd SECORE field workshop included not only the physical difficulty of working in the dark during the collection of gametes but also a number of other issues. These challenges included limited access to fresh seawater, inadequate laboratory conditions, and larvae collection net failure. Errors in transportation resulted in over 100,000 larvae being lost. Further difficulty arose over obtaining permits for the transport of endangered species to European nations. At public aquaria in the United States, coral specialists encountered problems with algal competitors, corallivores, zooxanthellae acquisition, and adequate nutrition for developing primary polyps and juvenile colonies. The problems concerning adequate settlement conditions were by far the greatest challenge to public aquaria specialists. The greatest long-term success was achieved by aquaria specialists with settlement tiles that had been cultured in established grow-out systems prior to the settlement of larvae. When tiles were allowed a succession of algal growth, primary polyps were settled onto tiles with sufficient coralline algae growth, rather than competitive filamentous algae. Interestingly, larvae would still settle onto tiles with no coralline algae or tile with competitive algae, however, coral polyps on tiles cultured with coralline algae faired the greatest because competition with filamentous algae was limited.

The 3rd and 4th SECORE workshops in Rincón, Puerto Rico sought to make improvements to the challenges encountered during the 2nd SECORE workshop. Off-season planning for SECORE included improving larvae culturing, primarily through improving water quality. The first priority for SECORE was to change the field laboratory site. In 2006, workshop participants traveled significant distances from gamete collection sites to larval culturing facilities, perhaps exposing larvae to unnecessary stress. Therefore, SECORE rented properties adjacent to *A. palmata* populations and gamete collection sites, which allowed for the construction of a temporary culturing system with access to relatively clean seawater as well as housing for participants. SECORE participants designed a sophisticated flow-through system using natural seawater to limit the time commitment necessary for the care of collected developing larvae (See Figure 6). Special containers called "kreisels" were also designed to help with optimizing larval development. The kreisels housed developing larvae and kept the larvae in motion with four seawater inputs with adjustable flow and a center input line (See Figure 7 and 8). Seawater exited the container via openings in the bottom of the container covered with 250μL mesh. Other major achievements included cryopreservation of the first Acroporid sperm, extraction of native zooxanthellae for enhanced coral rearing in captivity and adoption of a protocol for public aquaria concerning the husbandry of *A. palmata*.

Figure 7 and 8. Above, larval rearing flow-through system. Below, larval rearing kreisel with larvae. Photographs by Andrew Hinrichs.

In 2009, SECORE returned for the 5th workshop in Puerto Rico. SECORE increased cryopreservation banking and methods for larvae, increased genetic crossing experiments and began a preliminary five-site field restoration experiment.

SUMMARY

The achievements of the SECORE project over the past five years can be summarized by a review of the production data. From 2006 to 2009, SECORE has cultured over 2.5 million *A. palmata* larvae, transported 900,000 larvae with 90% survival, and achieved 5-36% settlement rate and 5-30% survival at three months in captivity. The settlement rate and survival rate achieved by SECORE members is 1,000 times greater than the natural settlement rate. Survival is also 1,000 times greater than survival for juvenile colonies in the Caribbean. To date, there are 2,000 living colonies of *A. palmata* that were captured during mass spawning events in Puerto Rico and developed with *ex situ* strategies.

FUTURE RESEARCH

SECORE is currently in the construction phase of a seaside cultivation facility in Curacao, Netherlands Antilles. This facility will serve as a permanent location for *ex situ* culturing and rearing of *A. palmata* larvae. Furthermore, this facility will enable SECORE scientists and members to develop protocols for the transplantation of *ex situ* reared coral onto degraded Caribbean reefs. SECORE will also explore other research interests such as investigating the flexibility of zooxanthellae symbionts, determining potential population bottlenecks and solutions, developing coral stem cells, identifying bacterial dynamics as settlement cues, increasing the genetic banking of eggs and larvae, as well as identifying resilience in captive and field coral colonies. SECORE strategies for *ex situ* coral propagation and future research developments are expected to lead to a greater understanding of factors that contribute to future success in preserving one of the earth's most diverse ecosystems, the coral reef.

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