Developing community-accessible methods of increasing coral reef resilience through selective coral breeding programs.

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Abstract: Techniques for active coral restoration using asexually derived fragments (cloning) to stock artificial reefs and nurseries have been well described and accepted over the last several decades. Somewhat surprisingly, however, these programs often do not include long-term monitoring, and do not address issues of decreased genetic and species diversity in resulting 'farmed reefs.' Recent techniques utilizing the culturing of coral larvae have been developed, but primarily have been applied at the academic level, and very little practical information has emerged for guiding local reef managers. Potentially, the culture of coral larvae to create feedstocks of corals with higher genetic diversity than the parent reefs is possible, but without more lay-accessible "handbooks", the benefits of such programs in increasing coral resilience cannot be realized. The program outlined in this paper was implemented on the island of Koh Tao from 2010-2014, and has focused on developing low technology methods for community-based reef managers to effectively increase the genetic diversity of farmed reefs. Moreover, the techniques are amenable to "selective breeding" of corals to accelerate adaptation to changing conditions and repopulating corals in degraded areas. During the period of the Koh Tao program, gametes from dozens of coral colonies from the hard coral *Goniastrea* have been fertilized and reared over three annual spawning episodes, and are growing to transplantable size on mid-water nurseries. The outcomes from this trial show that community managers can utilize such programs to overcome many of the inherent problems in using cloned coral fragments for restoration purposes.
Keywords: Coral Restoration, Larvae Culturing, Community Management of Reefs

Introduction

The major direct stresses and disturbances to coral reefs are well documented and include; pollution, sedimentation, nutrient enrichment, structural damage, over-extraction or use, and habitat destruction (Bruno & Selig 2007, Wilkinson 2010). Mass coral bleaching due to ocean warming has become more frequent and severe over the last 30 years, decreasing reef abundance and diversity around the globe (Brown 1997, Hoegh-Guldberg 1999). Due to both localized and global threats, many reef areas have become so depleted that it is unlikely they can recover on their own, or will take such significant amounts of time that dependent economies will be severely impacted (Wilkinson 2008, Bruno and Selig 2007).

Most reef management techniques implemented over the last 20 years have been focused on the designation of Marine Protected Areas (MPAs), and other passive management techniques. Despite all management efforts implemented to date, global reef decline has risen from 1% per year before 1997, to 2% between 1997 and 2003 (Bruno & Selig 2007, Rinkevich 2008). The results show that purely passive or protective measures are quite often insufficient on their own, and that active restoration and disturbance mitigation projects are vital for the survival of coral reef ecosystems and related economies (Edwards & Gomez 2007)

Most mainstream coral reef restoration projects (particularly those available to community based programs) have been focused on increasing reef structure through artificial reefs, increasing coral abundance through asexual propagation (cloning), or transplanting of corals through various nursery and gardening techniques. Although these asexual techniques are well described and accepted, recent data indicates that their benefits are primarily short-term, and long term effects are generally neutral or negative (Shearer *et al* 2009, Precht *et al.* 2005), and will almost certainly decrease the long term resilience and adaptability of the reefs (Baums 2008). Inherent problems stemming from the use of asexually derived coral fragments in restoration include the ecological costs of removing colonies from donor reefs (Clark and Edwards 1995), an (inappropriate) focus on fast growing and easily-propagated species at the expense of coral diversity (Edwards and Clark 1998), genetic bottlenecking and reduced genetic variability of restored reefs, and reproductive failure due to inbreeding depressions (Baums 2008).

Recent innovations and techniques involving the culturing of coral larvae can allow reef managers to produce sexually derived restoration feedstocks, without impacting donor reefs (Guest *et al.* 2010). Concurrently, the methods also reduce generation times, increase reproductive success, increase genetic or genotypic variability, and reduce genetic 'bottle-necking' or inbreeding depression, and may expedite the adaptation of corals to climate change through inter-specific hybridization (Willis *et al.* 1997). Although there is strong evidence pointing to the role of horizontal gene transfer in ability of corals to respond to climate change (Van Oppen *et al.* 2003), to date no literature is available providing guidelines for the use of hybridization techniques in coral restoration (Hatta *et al.* 1999, Vollmer & Palumbl 2002).

The basic techniques for coral gamete collection and culturing have been established and described for over a decade, however there are few community level restoration programs, that we are aware of, that are utilizing the techniques for active restoration. In Thailand, there are only two other known programs using coral larvae for restoration based projects (Chavanich and Viyakarn 2011). The main barrier preventing local reef managers from utilizing and further developing these techniques is a lack of information and guidance to non-scientists about how to rear and maintain coral larvae using locally available materials for use in reef rehabilitation and disturbance mitigation.

Between 2010 and 2014, a coral larval culturing project was undertaken on Koh Tao, Thailand, to examine low cost, low-tech ways to assist community level programs in creating selective coral breeding programs for active restoration. Through this project, coral colony resilience was observed during and subsequent to the large scale mass bleaching event of 2010. That information was then used to modify versions of published methods and techniques to work in the local context. The program evolved over three years, seeking to improve the techniques and develop more cost effective and efficient materials and methods to suit the community-based reef management model of the island. Here we outline some of the key lessons learned during that process.

Methods and Materials

Study Site

Koh Tao is a 19km^2 island which is host to some of the most abundant and diverse reefs in the Gulf of Thailand; these reefs attract between 300,000 and 400,000 visitors per year (Larphnun *et al.* 2010). Over the last decade, the island has become the center of SCUBA Diver Training for SE Asia, and boasts the second largest dive industry in the world – responsible for fully one-third of all the PADI certification issued globally in 2009 (Wongthong and Harvey 2014). Rapid growth and uncontrolled development have placed major stress on the terrestrial and marine ecosystems of Koh Tao, with a 50% reduction in natural forests by 2009 (Weterings 2011), and coral coverage reduced by 17% in the years from 2001 to 2006 (Yeemin 2006). On top of direct chronic stresses associated with local anthropogenic activities, the island's reefs were severely impacted by the mass coral bleaching events of 1998 and 2010 (Chavanich *et al.* 2012, Yeemin *et al.* 2012).

Management of marine areas on Koh Tao historically had been either absent or ineffective (Garces 1992, Wongthong and Harvey 2014), until the creation of the Save Koh Tao Community group in 2008. This local management model has achieved some successes in protecting and restoring reefs through education, distributed reef monitoring programs, regulations and zoning, creation of alternative dive sties, installation of artificial reefs, coral predator removals, and coral nursery and transplantation techniques. The presence of trained local reef managers already involved in reef restoration using asexual propagation techniques for over 6 years through the New Heaven Reef Conservation Program (NHRCP) significantly reduced the learning curve of techniques in this study.

Site and species selection

Surveys after the 2010 mass bleaching event found that the shallow reef of Chalok Ban Kao bay (3 to 6 meters depth) had the highest mortality rates around the island, with 64% by October of 2010. Due to the bay's high mortality rate and proximity to the culturing station, it was selected as the targeted collection and restoration site. Three collection sites within the bay were chosen which contained at least 6 reproductive-sized colonies of *Acropora spp.* and several Faviid species of different genera. The surviving mature colonies were widely dispersed, and comprised what were assumed to be three distinct breeding populations; i.e. far enough apart to have a low chance of natural cross fertilization in a mass spawning event (about 70-120 meters).

Prior to the spawning period, corals were examined at regular intervals of about two weeks for pigmented eggs through on-site histology as per (Kongjandtre 2010, Piromvaragorn *et al.* 2006). After the observation of strongly-pigmented egg bundles in coral samples, volunteer diver teams from the NHRCP were deployed nightly to observe the corals. Divers were supplied with cameras to record any reproductive activity and behaviors. A key component of the techniques outlined in this paper is that – unlike cloned-propagation techniques – no corals are harmed during the collection process; gametes are collected from the water column during natural spawning events.

Construction of culturing tanks

Coral larvae culturing and rearing units were constructed near the beach in Chalok Ban Kao. In 2010, the units were made of large plastic tubs. Regular water exchanges were carried out via siphoning through small diameter silicon tubing; volunteers collecting fresh sea water in buckets from above the reef (about 400m from the beach). Water changes using manual siphoning were tedious and not entirely effective. In 2013, culturing units were constructed using rendered cement and PVC standpipes allowing a continuous flow of water. The semi-closed system consists of 100 liter coral culturing tanks, a filtration tank, and a water treatment tank with macro-algae to strip excess nutrients from the water. 10-20% of the stored water was released from the sump tank every day and replaced with coarsely filtered fresh sea water pumped from the bay at high tide. The installation and operation cost in 2013 of the new culture system was about $3,000 USD.

Training local stakeholders and volunteers

Presentations about rehabilitation and coral spawning were made at monthly meetings of the Save Koh Tao Community Group over several months to increase awareness and encourage participation in the trial project. Volunteer divers from the NHRCP and other dive centers were given preparation in the form of three 60-minute lectures on the theory and practical requirements of this project. During the period leading up to spawning episodes, divers were briefed on the gamete collection procedure and practiced mock-spawning exercises in preparation for the rapid deployment of dive teams at the onset of spawning. Prior to spawning, individual colonies targeted for collection were marked using color-coded nylon rope

Gamete collection procedures were based on a modified version of Verimeij *et al.* (2006, 2009). In addition to the standard night diving equipment for SCUBA diving, each diver was equipped with a modified butterfly net to collect coral egg/sperm bundles as they are released from the target colonies, and red-filtered torches so that the divers could navigate safely without affecting spawning behavior. Volunteers were instructed to collect no more than 50% of the total reproductive output from any single colony, and to collect bundles from several colonies within each distinct subpopulation to maximize the genetic diversity of the collected gametes. The total cost of the gamete collection materials for the entire four year study was less than $600 USD. The nightly observation dives and the gamete collection dives were cost-neutral, through the use of a volunteer labor force.

Fertilization of coral gametes

After collection, gametes were transferred underwater to collection jars and brought to the dive vessel for fertilization according to modified version of the methods suggested by Heyward (1999). In order to maximize genetic variation of the resulting embryos, gametes collected were mixed with all those from colonies of the same species, allowing fertilization between normally isolated populations of corals in the same region. The density of gametes in any single fertilization container was kept to about 80-90% coverage of the water surface (as per Edwards 2010). Clumps or aggregations of eggs were gently separated using a Pasteur pipette or other small volume wash bottle. The gametes in the fertilization buckets were gently agitated by a continuous trickle of bubbles from a small airstone supplied by a battery-powered aquarium air pump to encourage mixing during the 1-1.5 hour fertilization period.

Culturing and Rearing of Coral Larvae

After fertilization, zygotes of each species were transferred to the culturing tanks for development; care was taken to exclude surplus sperm, which tends to break down and degrade water quality very quickly. Water was slowly circulated constantly through the tanks to maintain ambient physical conditions and water quality. Water changes were performed every 12 hours for the first 36 hours post-fertilization, using 50% of the volume of the sump tank (about 15% of the total culturing unit volume) each time. After the first 36 hours, the frequency of water changes was reduced to once per day (between 5-20% of the total water capacity).

The larvae in the culturing tanks were observed frequently by volunteers for color, size, and swimming behavior. After approximately four days, the larvae reach a settlement competent stage, when they are observed to swim down to the bottom of the tanks and explore the substrate (Vermeij *et al.* 2009). When this behavior was observed, artificial concrete settlement substrates — created by volunteers using a modified version of the 'Coral Peg' technique described by Omori (2009) — were introduced to the tanks along with dead coal fragments colonized by coralline algae obtained from the natal reef. The artificial substrates had been pre-conditioned by submersion at the natal reef for between 1-12 months (fabrication of the settlement plugs was part of the "engagement activities" used to familiarize volunteers with the concepts, and so production was staggered between cohorts of volunteers). The pre-conditioning allows the formation of a microbial biofilm on the substrate which enhances settlement success. Before being introduced to the tanks, the settlement substrates were cleaned of any macro-algae, tunicates, sponges, or other fouling organisms by scrubbing with a coarse brush. After two days, the water in the settlement tanks was completely replaced by fresh seawater, and the evacuated water taken out to the reef, where any larvae that had not yet settled had opportunity to colonize the natural substrates.

After settlement, spat were maintained in ambient conditions, under natural light regimes, for the remainder of the project. Fragments of living corals were placed into the tanks to provide a source of zooxanthallae, and coarsely filtered sea water was introduced to the system daily, containing zooplankton as a potential food source for the juvenile corals. Half of the juveniles were transported to a floating nursery near the parent reef at 1 month post-settlement. After an additional 2-6 months, the remainder were also moved to mid-water coral nurseries situated at a depth of 3-6 meters in either Ao Leuk Bay or Chalok Ban Kao Bay. Colonies at the nurseries are maintained and monitored by volunteers from the NHRCP on a monthly basis.

Results and Discussion

Coral gamete collection, fertilization, and rearing were carried out using volunteer teams of local reef managers in 4 of the 5 years between 2010 and 2014. The project was not carried out in 2011 because the bleaching event of 2010 apparently interrupted gametogenesis in the subsequent cycle; this is consistent with observations from other regions following mass bleaching events (Ward *et al.* 2000). During 2010 and 2012 the use of many of the techniques described in the scientific literature by non-professional teams and assistants proved difficult; moreover, the bleaching event in 2010 may have killed many of the newly-settled juveniles in our nursery. However, 1 colony of *Goniastrea* cultured in 2010, had reached a mean colony diameter of 9.2 cm by June of 2014 while growing on a mid-water coral nursery. Success was greater in subsequent years, with 6 colonies of *Goniastrea* from 2012, and 18 viable colonies from the 2013 project still surviving, as of June 2014. These numbers appear somewhat less than the survivorship described elsewhere in the literature; however, they are evidence that low-tech, low-infrastructure operations using volunteer labor can, with increasing certainty, successfully culture juvenile corals.

Modifications were made throughout the program, using locally available and inexpensive materials to improve success amongst the local volunteer teams. A series of presentations and a manual on *A Genetic Approach to Coral Restoration* (Scott, in press) was developed for use in training the teams for 2013 and 2014. Building on lessons learned in the first spawning events, diver teams proved to be more effective and there was little error in the collection and fertilization procedures of the project. In 2014, a team of 27 volunteer divers were engaged to determine the limits to scaling of this type of operation. Preliminary results from the 2014 project show that success may actually be two to three-fold higher than the 2013 project, suggesting that success is mostly limited to the logistics of coordinating larger teams, and that the techniques can be scaled to fit the application. Although initial results are promising, it may take a decade or more to fully quantify the benefits of such programs due to the slow growth and reproductive rates of corals. However, this project has advanced the development of coral culturing projects amongst local reef managers, training over 55 local reef managers and volunteer divers on Koh Tao in the techniques.

The success of this project suggests that the false paradigm of expensive and highly specialized techniques described for coral culturing in the scientific literature should be replaced, and the culturing techniques adapted for use by non-professionals with limited resources. The total cost of the project for the 4 years of implementation was about $5,200 USD, amounting to about $67 USD per coral colony for the 78 so far produced. Guest *et al*. (2014) suggested that culturing from larvae costs around $60 US per coral, but it should be noted that the largest cost in the Koh Tao project were for infrastructure: the culturing tanks, which are reused for each subsequent year's project, and can be amortized over several years. Thus, each year the total cost per coral produced declines; generating 50 coral colonies per year, the average cost per coral could be as low as $15-20 USD. This is quite comparable to the costs published for asexually produced coral colonies in other studies (Edwards 2010), and is likely to decline further as scale issues are addressed. Crucially, in this context, it should be noted that the notion of participating – not only in coral spawning events, but also in larval husbandry – is extraordinarily captivating for “eco-dive” tourists, and represents a huge potential market for dive operators. The use of paying volunteers actually makes reef rehabilitation cost-neutral for forward-looking community groups such as Save Koh Tao. These volunteers are a self-selecting group of skilled divers who are easily-trained and highly motivated, and are an opportunity for outreach and awareness that should be captured wherever possible.

Conclusion

In order to be effective, coral restoration programs must make a transition from the traditional methods of asexual restoration, to using an approach which factors in the genetic diversity of restored areas. Management programs with a focus to increase genetic variability, over short-term changes in abundance, have potential to assist in the adaptation of reefs and make coral restoration and protection efforts more effective. Encouraging hybridization between populations or species may also assist in increasing the amount of novel genetic material available, and allow the transfer of stress tolerant genes between populations or species (Van Oppen *et al*. 2003, Willis *et al.* 2006). Although described for over a decade, few reef managers are utilizing techniques of coral culturing or selective breeding for active restoration. More accessible techniques, and publications directed towards non-scientists, are necessary before the wide-spread use of such programs can be realized. Encouraging public participation and integrating coral restoration programs with courses on marine conservation, allows such projects to be primarily self-funded, and may even provide new industries to preserve the livelihoods of local residents (Scott and Phillips 2010).

This project suggests that local reef managers can use sexually produced feedstocks of corals in restoration to practically address coral reef resilience and adaptation. But, in order for this goal to be realized it will take the continued work of a dispersed system of professional researchers and local reef managers to develop these techniques further. If current rates of reef decline continue, the coral environments and their associated economies may be lost within the next century. With more scientifically informed techniques, and a shifted focus to adaptation and resilience, local reef managers may one day be able to preserve and sustain the use of coral reef ecosystems in the face of chronic local threats and climate change.

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