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ABSTRACT

Corals are important to coastal ecosystems and communities; therefore, they need to be protected and conserved. Sea surface warming, anthropogenic pollution, overfishing, and overdevelopment have been found to contribute to the bleaching, disease, and death of corals. Recent improvements in technologies and methodologies have increased the feasibility of propagating corals in captivity. The goal of this project was to continue to complete the beginning steps necessary to establish a coral nursery in Hilo, Hawai'i. On 8 October 2018, Matthew Connelly acquired the permit required to house coral fragments for the project. A 125gallon aquarium, plumbed to a 75-gallon sump, was set up to house the coral fragments at the Pacific Aquaculture and Coastal Resources Center (PACRC) in Keaukaha, Hawai'i. On 15 October 2018, the Department of Aquatic Resources (DAR) collected 216 coral fragments in Kailua Bay, Hawai'i. All fragments were transported from Honokohau Harbor in Kailua Kona, Hawai'i to the established system at PACRC. Water parameters of the system were monitored consistently by all project members. Due to unforeseen difficulties and unavoidable events, the coral fragments received by the Coral Propagation Project did not survive. Despite all the issues encountered, the corals were kept alive for 83 days and a great deal of experience and knowledge was obtained. This experience has only motivated me and my project members to grow this project to the point where we have a fully established coral nursery at PACRC.

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INTRODUCTION

Corals are colonial organisms that create the framework of coral reefs, which host a wide array of biological diversity rivaling any other marine ecosystem on Earth. Corals use symbiotic photosynthetic dinoflagellates, called zooxanthellae, which provide the corals with glucose. (Hoegh-Guldberg 1999; Wild et al. 2011). Due to these zooxanthellae, coral reefs are an important source of atmospheric oxygen (Wild et al. 2011). Additionally, coral reefs provide protection from storms and tsunami to many coastal cities, participate in extremely efficient nutrient cycling, and sustain industries including global fisheries, medicine, and tourism (Friedlander et al. 2008; Wild et al. 2011; Maynard et al. 2016; Tynyakov 2017). In Hawai'i, coral reefs were historically and still are important to the Hawaiian culture, as the coral polyp was the first organism to arise in the Kumulipo, the Hawaiian Creation Chant (Friedlander et al. 2008; Maynard et al. 2016). Additionally, coral reefs create habitat for Hawaiian fish and invertebrate species, which are an important food and economic resource for many Hawai'i residents (Friedlander et al. 2008; Maynard et al. 2016).

Corals are vulnerable to environmental changes in temperature, salinity, turbidity, sedimentation, and chemical fluctuations, as well as to anthropogenic stressors (Smith et al. 2016). These can be detrimental to coral health and can result in bleaching, disease, and death (Santavy et al. 2005; Maynard et al. 2016; Couch et al. 2017). Coral bleaching is largely a defense mechanism used by corals to conserve energy when environmental stress is placed on them (Santavy et al. 2005; Couch et al. 2017). A coral expels its symbionts, resulting in a bleached or pale appearance (Maynard et al. 2016; Couch et al. 2017). In some cases, if the environmental conditions quickly return to normal, corals can recover and zooxanthellae may return; however, if too much time passes, the coral colony can die (Santavy et al. 2005; Couch et al. 2017). Large temperature fluctuations have been found to initiate extensive bleaching events, like those observed in Hawai'i in 2014 and 2015 (Maynard et al. 2016; Couch et al. 2017). In South Kohala, on Hawai'i Island after the 2015 mass bleaching event 55-99% of reefs surveyed experienced coral bleaching and mortality (Maynard et al. 2016). Furthermore, in 45 countries and 5 United States territories, overfishing was found to threaten approximately 55% of coral reefs (Wear 2016). With populations of coastal cities increasing steadily over time, development of coastlines and pollution associated with it has been correlated with the decline of coral reefs (Wear 2016). Threats to corals reefs are well documented and possible solutions are being put into practice to protect healthy coral reefs and restore impacted coral reefs.

Mitigation of threats against coral reefs, due to large temperature fluctuations, overfishing, and coastal development, is imperative to the conservation of corals (Hesley et al 2017). Many methods, from restoration of natural reefs, establishment of artificial reefs, or changes in management strategies, have been used in this endeavor. An important method for restoring natural reefs involves propagation and out-planting of corals (Barton et al. 2015; Hesley et al. 2017). Coral nurseries, both *in situ* and *ex situ*, are important to this type of restoration (Lirman et al. 2010; Boch & Morse 2012; Barton et al. 2015; Hesley et al. 2017). *In situ* nurseries generally involve the use of racks or "coral trees" that are anchored to the sea floor in areas with low wave action (Lirman et al. 2010; Boch & Morse 2012; Barton et al. 2015; Hesley et al.

2017). Ex situ nurseries house corals in recirculation tanks which keep water parameters constant and where other environmental fluctuations of light, ambient temperature, water movement, and turbidity can be kept to a minimum (Lirman et al. 2010; Boch & Morse 2012; Barton et al. 2015; Hesley et al. 2017). Coral nurseries propagate corals via asexual or sexual reproduction. Asexual reproduction is more commonly used in coral propagation and is executed by fragmenting an existing coral head (Johnson et al. 2011; Barton et al. 2015). In ex situ nurseries, sexual reproduction has been found to occur naturally in tanks without facilitation; however, nurseries are able to induce spawning in corals if certain environmental factors, including the lunar cycle, diel cycle, and temperature are altered (Craggs et al. 2017). Coral propagation is important to the conservation and restoration of declining coral populations of specific coral species such as Acropora cervicornis (Johnson et al. 2011; Hesley et al. 2017). Boch & Morse (2012) found that out-plantings using coral fragments of Acropora spp., produced sexually or asexually, proved to be a viable and cost effective method for reef restoration. Hawaiian coral species are susceptible to the same threats against coral reefs, the goal of the Coral Propagation Project is to aid in propagating coral fragments.

The creation of an *ex situ* coral nursery is the aim of the Coral Propagation Project, located at the Pacific Aquaculture and Coastal Resources Center (PACRC) in Hilo, Hawai'i (Figure 1). PACRC is a facility operated by the University of Hawai'i at Hilo (UHH), which specializes in aquaculture and aquatic research. This facility contains large pumps that provide seawater from Hilo Bay to the projects on the property. The Coral Propagation Project is a Marine Option Program (MOP) project that was created by Michelle Nason. The goal of this project is to collect coral fragments from the wild that are broken off mother colonies due to large storm events, boating mishaps, and other anthropogenic influences that result in the fragmentation of coral, and propagate them in a closed-system. Local species that may be good candidates for propagation are *Porites spp.*, *Meandrina spp.*, *Montipora spp.*, and *Pavona spp.* (Couch et al. 2017). Fragments grown in this nursery will be available for a variety of purposes including research, educational outreach, and coral reef restoration via out-planting.

The main focus of this MOP project was to build a stand-alone educational display piece. The purpose of this educational display piece to serve as a way of explaining the purpose of the Coral Propagation Project. Historically, the use of interpretative signage has been very effective and useful in public aquariums, national parks, and zoos (Packer & Ballantyne 2010). This type of signage, if done correctly, can efficiently inform visitors about the purpose of a project, life histories of organisms, rules and regulations, and the span and scope of a project or organization (Packer & Ballantyne 2010). Visitors to public aquaria and marine parks had greater memory retention of what was on the signage when threats to the organisms were emphasized and solutions were proposed (Ballantyne et al. 2011). Additionally, signage in close proximity of the organisms in each exhibit increased interest and memory retention (Ballantyne et al. 2011). This type of educational outreach is important in informing community members about the importance of coral conservation and restoration.

Objectives and direction of the project shifted after an anchoring event that occurred in Kailua Kona, Hawai'i. Therefore, the new objectives of this project were to establish a 200-gallon (gal) temporary holding tank for coral fragments, acquire and transport coral fragments from the Department of Aquatic Resources (DAR) office, and maintain the temporary holding system.

METHODS Project Location

The location of this project was at PACRC in Hilo, Hawai'i (Fig. 1). At PACRC, large pumps provided seawater, from Hilo Bay, to the project. All incoming seawater was filtered using Ultra Violet and Ozone filtration.

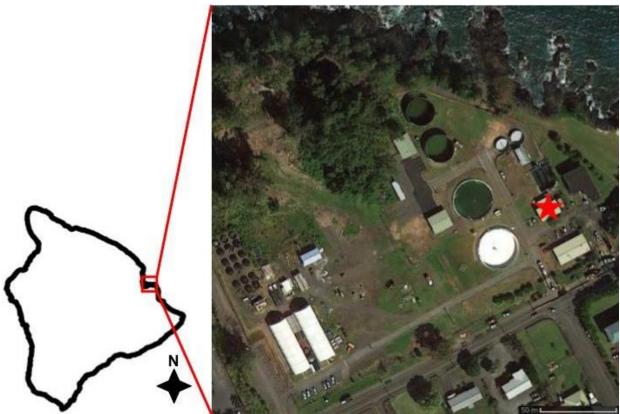


Figure 1. Satellite map showing the location of the project (red star) at PACRC (Google Maps 2019).

Establishment of Educational Display

At PACRC, a wooden stand was outfitted with one 75-gal glass aquarium, one 20-gal glass aquarium, and one 50-gal plastic sump. This stand was placed on a concrete slab, adjacent to the output well for PACRC (Fig. 2). The two aquariums on the stand were plumbed to the 50-gal plastic sump using 15 feet (ft) of ¾ inch (in) polyvinyl chloride tubing (PVC), 8 x ¾-in 90° PVC elbow fittings, 3 x ¾-in three way PVC tee fittings, 1 x ½-in 90° PVC elbow fitting, 1-ft of ½-in PVC tubing, 3 x 1-in 90° PVC elbow fittings, and 10-ft of 1-in PVC tubing. The plumbing for the return, from the sump to the aquariums, was assembled using the ¾-in PVC tubing/fittings and 4-ounce NSF Purple Primer with Oatey 30246 PVC Regular Cement.

For the drainage from the aquariums to the sump, the 10-ft of 1-in PVC tubing was cut in

half. One 5-ft piece was attached directly to the drains for the two aquariums using 2×1 -in 90° PVC elbow fittings and the 4-ounce NSF Purple Primer with Oatey 30246 PVC Regular Cement. The remaining 5-ft of 1-in PVC tubing was cut in half again and used as standing drain pipes for the 75-gal aquarium.

The ¾-in PVC tubing was cut into the following segments: 4 x 1-ft pieces, 2 x 3-in pieces, 2 x 1.5-ft pieces, and 3 x 2.5-ft pieces. These segments were cemented together using the 8 x ¾-in 90° PVC elbow fittings, 3 x ¾-in three-way PVC T-shaped fittings, and the 4-ounce NSF Purple Primer with Oatey 30246 PVC Regular Cement in the configuration shown in Fig 2. Once all cemented together, the plumbing could hang on one wall of the 75-gal aquarium. The end that was outside of the aquarium was attached to the EcoPlus Eco 1584 Fixed Flow Submersible/Inline Pump using 3-ft of 1-in clear PVC tubing. The pump returned water from the sump to the aquarium. When the system was turned on, water flowed from the aquariums to the sump, then back to the aquariums. This system was completed on 20 September 2018; however, a stable electrical supply was not available for the system to be connected to.



Figure 2. Wooden stand with 75-gallon and 20-gallon glass aquariums with plumbing leading to 50-gallon plastic sump (not pictured).

Establishment of Temporary Fragment Holding Aquarium System

A 125-gal aquarium was placed on a stand and was plumbed to a 75-gal sump. The system was placed under a covered pavilion area at PACRC (Fig. 3). Similar to the system wooden stand display system, this aquarium was plumbed to an external sump which contained two Aqueon submersible heaters, a DOC Skimmer 9410DC protein skimmer, and one 1800 gal/h PondMaster Magnetic Drive Utility Pump. This closed system allowed the control of water parameters and prevented the introduction of unwanted pests and diseases. A refractometer was used to test the salinity of the system. Water coming from the aquariums, through a custom-built overflow box, was run through mechanical filtration, via 150-micron filter socks. The DOC Skimmer 9410DC protein skimmer was used to remove dissolved dead organic matter from the water in the system, that couldn't be removed by the filter socks. The two heaters ensured that

the water was always at the correct temperature and minimal temperature fluctuations occurred. The 1800 gal/h PondMaster Magnetic Drive Utility return pump was attached to 3.5-ft of 1-in clear PVC tubing, which was clamped onto a two-way nozzle made from 1-in PVC and used to return water from the sump to the 125-gal aquarium.

In addition to the external sump, two 110-W Aqua Clear Power Filters were used as extra filtration for the system. Within the aquarium, two Koralia movement and circulation pumps were used to simulate natural ocean currents. Three coral fragment racks, made by Michelle Nason, were placed in the aquarium. The 125-gal aquarium was covered using three glass covers that came with the aquarium. On the covers, two Aquasun LED HO lights and two Aquasun T5 HO 24" lights were placed. These lights were set to a 12-h light cycle to simulate normal light conditions. The system was established on 15 October 2018 and was operated until 28 December 2019.



Figure 3. 125-gallon glass aquarium, plumbed to 75-gallon plastic sump (not pictured), at PACRC.

Acquisition of Coral Fragments

The permit to collect, house, and grow corals was written by Matt Connelly and submitted to DAR on 20 March 2018. On 8 October 2018, the permit was approved by the DAR. On 15 October 2018, DAR collected 216 coral fragments, using open circuit scuba diving, in response to an anchoring incident that occurred the previous week in Kailua Kona, Hawai'i. Fragments were stored in clear plastic tubs by the DAR response divers, then brought to the surface. On the same day, the coral frags were retrieved from the DAR boat. The tubs containing the fragments were placed in two Aussie Box coolers with enough seawater to cover the tubs and transported to PACRC.

Once at PACRC, Scott Hardman, Matthew Connelly, Michelle Nason, and I (the Coral Crew) unloaded the coral fragments. A Seachem Reef Dip was prepared to ensure that the new coral fragments were free of parasites and hitchhikers (Fig. 4). Each coral fragment was placed in the Seachem Reef Dip for 15-min and then glued to a ceramic tile piece using super glue.

Once the super glue had set, the coral was logged with number and species name. Before it was placed in the tank, the fragment was dipped in fresh seawater to wash off the Seachem Reef Dip. Finally, the Coral Crew put each fragment onto the fragment racks in the 125-gal system. With a total of three species; *Porites lobata*, *Porites rus*, and *Porites compressa*, each coral fragment needed to be placed in the tank based on water flow rates and light exposure requirements (Fig. 5). Specifically, *P. compressa* and *P. rus* were gotten from the deeper areas; thus, they were put in area of lower water flow and light exposure. This process took 10-h in total with all Coral Crew members working as fast as possible to reduce the amount of stress on the fragments.



Figure 4. Coral fragments sitting in the Seachem Reef Dip before being glued to tile and logged.



Figure 5. Final setup of the temporary coral fragment holding aquarium with all 216 coral fragments in the tank.

Maintenance of Temporary Coral Fragment Holding Aquarium

Water temperature, pH, and salinity of the 125-gal system were consistently monitored using a YSI Pro2030 and a refractometer. The water temperature was kept at 23-24° C using the heaters in the sump and a Neptune Systems Apex Controller System. Salinity was held constant at 35 parts per thousand (ppt) and the system was replenished with deionized (DI) freshwater when salinity rose too high. To monitor the nitrates, nitrites, ammonia, phosphates, alkalinity, and calcium levels, API Reef and Saltwater Masterkits were used. Nitrates, nitrites, ammonia, and phosphates were kept as close to zero as possible. Alkalinity was kept between 10-17 degrees of carbonate hardness (dKH). If the nitrates, nitrites, ammonia, phosphates, or alkalinity were too high, a water change of approximately 40-gal was completed using the water at PACRC mixed with Instant Ocean Reef Crystals. Calcium was maintained between 410-420 parts per million (ppm). If calcium levels dropped below this, the tank was dosed with Seachem Reef Calcium. Doses varied because the amount needed to get the system back to the appropriate levels was calculated each time using the formula on the Seachem Reef Calcium bottle. The coral fragments were fed on Monday and Friday using 5 ml/gal of Seachem Reef Zooplankton and Seachem Reef Phytoplankton. The coral fragments were kept in the system from 15 October 2018 to 25 December 2018.

DISCUSSION

The aim of this project has changed significantly since its inception. With multiple road bumps along the way, the main goal of the project was to build an educational display. A reliable electrical supply was not available, which prevented the educational display system from running. Without a stable supply of electricity, the equipment for the educational display could not run for extended periods of time. While this issue was being addressed, the focus of the Coral Crew shifted when DAR requested that a system be set up to house corals. To set up a temporary holding system, components from the educational display system were used to establish the 200-gal system. The sump, return pump, and PVC fittings were recycled from the educational display system. The coral fragments and the system required constant maintenance and attention from the Coral Crew.

Once the 125-gal system was set up, some problems arose and require immediate action. One issue was the presence of diatoms in the seawater used to fill the tank. Diatom blooms quickly began to multiply at a fast rate after the first month. When the diatom blooms occurred, water changes were done with clean water and all of the coral fragment tiles were scrubbed to remove as many diatoms as possible. The bloom caused a large amount of stress on the coral fragments and it took a few days to rebalance the system. After the diatom bloom and water change, the alkalinity began to rise much higher than the optimal levels. Despite completing many water changes the alkalinity kept rising. After the third water change, we realized that the freshwater they were mixing with the Instant Ocean Reef Crystals had very high levels of alkalinity. Thus, DI water mixed with Instant Ocean Reef Crystals was used instead and the alkalinity issue was resolved. The tank was stable for the rest of the time that the system was running, but on 10 December 2018 the system overflowed and the Neptune Systems Apex Controller System was lost. This was a very expensive piece of equipment and cost the project 1000 USD.

The anchoring incident, transport of the fragments, dipping, gluing, diatom bloom, and

alkalinity spike proved to be too much stress for the coral fragments. On December 25th 2018, the last living coral fragment died. The dead fragments were removed from the tank and stored for later use in a calcium reactor. All of the equipment for the 125-gal system was unplugged and disassembled. Though the situation was not ideal, using the experience we gained from March 2018-January 2019, plans for future projects and systems are in place.

Broader Impacts

The immediate impact of this project was to move UHH closer to having a fully-functioning coral nursery. On a broader scale, this MOP project could contribute to the future restoration of Hawai'i's coral reef ecosystems. Restoration could be accomplished by outplanting efforts using coral fragments grown by the Coral Propagation Project. Reef restoration using outplanted coral fragments has been found to be successful, using *Acropora spp*. (Johnson et al. 2011; Tynyakov 2017; Hesley et al. 2017). My project and the Coral Propagation Project could achieve the same results for local species, including *Porites spp.*, *Pocillopora spp.*, *Montipora spp.*, or *Pavona spp.* These local species in, areas heavily impacted by the 2014-present global bleaching events, could be conserved and restored (Couch et al. 2017). This facility could provide research opportunities, for postgraduates, graduates, and undergraduates, on local coral species.

Future Studies

In the months of April-May 2019, DAR has proposed that the Coral Propagation Project and the Coral Crew to assist in rearing coral larvae. This will be an excellent opportunity for project members to work and gain more experience with coral propagation and to attract attention to the project. New systems are in the process of being established and new project members are being recruited to help with the project. Exciting things are in store for this project and we are one step closer to having a functioning coral nursery.

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