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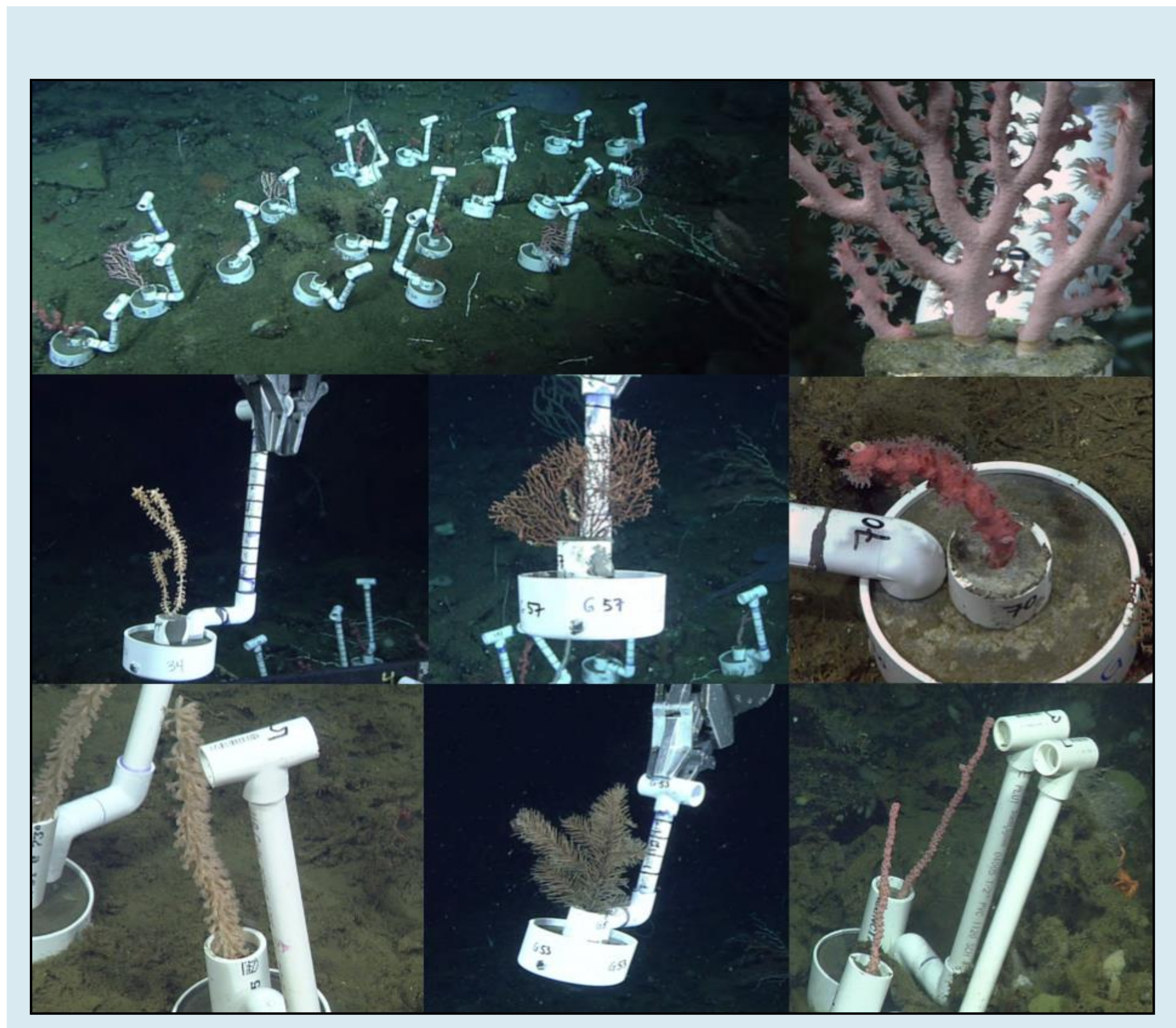
Guide to Translocating Coral Fragments for Deep-sea Restoration



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Cover Photo:
The seven coral species investigated for developing deep-sea coral restoration methods at Sur Ridge, Monterey Bay

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ABSTRACT

Corals in rocky deep-sea environments are foundation species postulated to enhance local diversity by increasing biogenic habitat heterogeneity and enriching local carbon cycling. However, deep-sea corals are highly vulnerable to disturbances (e.g., trawling, mining, and pollution) and are threatened by expansive changes in ocean conditions linked to climate change (e.g., acidification, warming, and deoxygenation). Once damaged by trawling or other disturbances, recolonization and regrowth of deep-sea corals may require centuries or longer, highlighting the need for their stewardship. To this end, the sustainability of deep-sea corals may be enhanced not only by protecting existing communities, but also by repopulating disturbed areas using active restoration methods. We recently reported one of the first studies to explore applied methods to restore deep-sea coral populations by translocating coral fragments of multiple coral species using translocation modules (Boch et al. 2019). Branches of deep-sea corals were collected by remotely operated vehicle (ROV) from 800–1300 m depth off central California and propagated into multiple fragments at the surface. These fragments were then attached to translocation modules ("coral pots") using two different attachment methods and placed in the same habitat to assess their survivorship (n=113 total fragments, n=7 taxa, n=7 deployment groups). Survivorship per year ranged from 0 – 100% depending on coral taxon and type of attachment method. Given relatively high survivorship among 5 out of 7 taxa studied, this report provides a more detailed step-by-step guide for fabricating coral translocation modules and for processing coral fragments from multiple taxa for deep-sea coral translocation. New survivorship data are provided as well, from new observations of translocated corals since the original publication, along with new insights from additional efforts focused on *Sibogagorgia cauliflora* husbandry.

KEY WORDS

deep-sea coral restoration, *Corallium* sp., *Lillipathes* sp., *Swiftia kofoidi, Keratoisis* sp., *Isidella tentaculum, Paragorgia arborea, Sibogagorgia cauliflora,* translocation module for coral fragments

INTRODUCTION

Human impacts in the deep sea are increasing from direct extractive activities (e.g., fishing and mineral extraction), pollution (e.g., oil spills and trash), and expansive changes in ocean conditions linked to anthropogenic climate change (e.g., ocean acidification, warming, and deoxygenation). While the integrated impacts of human activities in the deep sea remain largely unknown, there is clear evidence that bottom trawling for fishes and invertebrates alone is leaving a global footprint from the nearshore to >1000 m depth (Amoroso et al. 2018). In the Pacific region, previous reports have shown that fishing gear around deep-sea coral aggregations can cause significant damage to deep-sea communities, including bubblegum and bamboo coral populations (Rooper et al. 2017; Salgado et al. 2018). In the Gulf of Mexico, catastrophes such as the 2010 Deepwater Horizon oil spill also demonstrated that an oil spill from a single platform can have far-ranging effects, spanning from ecological impacts in the deep sea (White et al. 2011) to economic depression in adjacent coastal communities—with the full potential impacts still unknown (McCrea-Strub et al. 2011; Sumaila et al. 2012). Although the Magnuson-Stevens Fishery Conservation and Management Act (2006) provides discretionary authority to regional fishery management councils to minimize negative impacts to essential fish habitats within the United States Exclusive Economic Zone, the slow rates of recovery for some damaged deep-sea assemblages suggest that active restoration efforts may be beneficial.

We focused on translocating deep-sea coral fragments from multiple coral taxa as a first step toward understanding the feasibility and facilitation of deep-sea coral recovery in the Pacific region for several reasons. Enhancing coral reef recovery in shallow reef systems via translocation or transplanting coral fragments is proposed to be more advantageous than using sexual propagation methods due to the cost-effectiveness and requirements for technical knowledge (Jaap 2000; Bowden-Kerby 2001; Epstein et al. 2001; Spieler et al. 2001; Rinkevich 2008; Edwards et al. 2010; Villanueva et al. 2012; Barton et al. 2015). That is, propagation of corals by harnessing gametes or larvae requires knowledge of coral biology and culturing that is more complicated than the knowledge required for translocation of coral fragments. For example, the propagation of *Acropora* sp. corals from shallow water reefs requires knowledge of the timing of coral spawning, collection and husbandry of gametes, procedures for fertilization and larval development, suitable larval settlement surfaces, optimal timing of larval exposure to settlement cues, and the methods for and timing of settling recruits onto a natural reef (Boch and Morse 2012). Horoszowski-Fridman et al. (2011) investigated the reproductive output of transplanted coral fragments versus natural coral colonies and concluded that transplanting nursery-grown colonies of *Stylophora pistillata* resulted in better reproductive capacities than natural colonies. Various attachment strategies for

scleractinian coral fragments have been reviewed and discussed (Barton et al. 2015) and insights from in situ experimental studies with shallow water gorgonians (20-25 m) are also available (Lasker 1990; Linares et al. 2008a, 2008b). Overall, the survival of both fragmented and sexually-propagated corals is lowest during the first post-transplant year and higher for transplanted fragments than sexually-propagated corals (Epstein et al. 2001; Boch and Morse 2012). In more than three decades of shallow water coral restoration research, survivorship of asexually-propagated corals has typically ranged from 30–40% after the first year in situ.

Studies of deep-sea restoration methods remain limited, but the initial insights show some promise. Restoration experiments using the deep-sea corals *Lophelia pertusa* (~500 m depth) and *Oculina varicosa* (~60-120 m depth) have been somewhat successful in the Gulf of Mexico (Koenig et al. 2005; Brooke et al. 2006; Brooke and Young 2009). In particular, Brooke and Young (2009) found that fragments of *Lophelia pertusa* corals attached to a polyvinyl chloride (PVC) transportable module could result in high survivorship up to 13.5 months. In the Mediterranean ecosystem, Montseny et al. (2019) showed that the gorgonian *Eunicella cavolini* caught as bycatch in trammel nets could be re-translocated with high survivorship (87.5% at 85 m depth after 1 year) using fragments and whole colonies attached to steel structures with epoxy putty (Corafix SuperFast, GROTECH®).

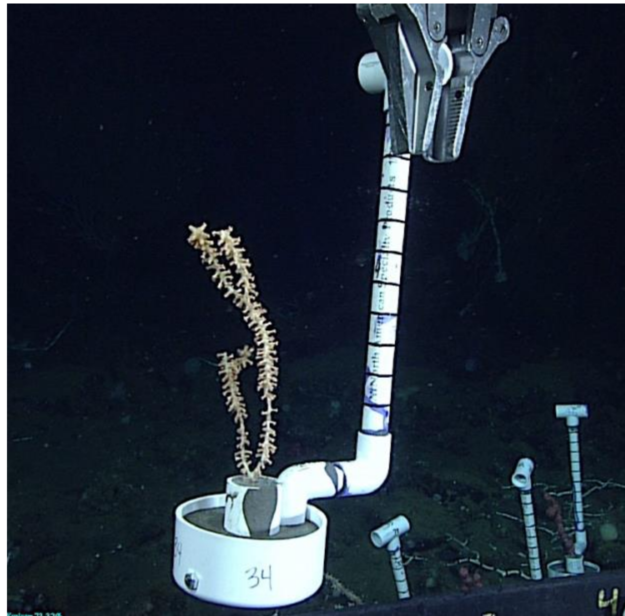
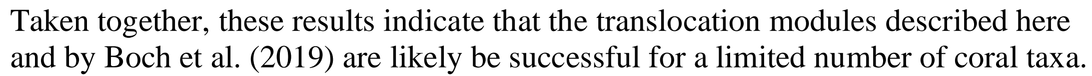


Figure 1. Deep-sea coral fragment transport module ("coral pot v.2") from Boch et al. (2019). Two versions of coral fragment translocation modules were evaluated, with this version resulting in higher survivorship for some of the coral taxa tested. Pictured is a *Keratoisis* sp. coral fragment being deployed by the ROV *Doc Ricketts* at Sur Ridge, California (approximately 1200 m in depth). Photo: MBARI

In our study of deep-sea restoration methods at Sur Ridge, California (~1200 m depth), we found that fixing coral fragments by "loosely" placing them on a PVC base resulted in total mortality of fragments deployed within a year (Boch et al. 2019). In contrast, fragments of corals (*Corallium* sp., *Swiftia kofoidi*, and *Lillipathes* sp.) attached to transportable PVC modules using a fast setting cement patcher (Fig. 1) resulted in up to 100% survival after ~3 years. Lower survival (0-50% after 3 years) was measured for *Keratoisis* sp. and *Isidella tentaculum*. Softer-bodied corals such *Paragorgia arborea* and *Sibogagorgia cauliflora* did not survive over 3 years, suggesting a need for alternative transportable modules and attachment strategies.



Taken together, these results indicate that the translocation modules described here and by Boch et al. (2019) are likely be successful for a limited number of coral taxa.

Although the PVC module method for translocating deep-sea corals may not be suitable for all coral taxa, it may be a useful approach for coral restoration efforts in some locations. In this report, we describe the coral translocation methods presented in Boch et al. (2019), including more details concerning the fabrication of coral pots and methods for fragment attachment. Updates on the survival of translocated corals are also reported, and the results from a pilot study investigating different *Paragorgia arborea* attachment strategies are discussed. Initial insights from the Monterey Bay Aquarium deep-sea coral husbandry program are also discussed to further inform efforts dedicated to *Sibogagorgia cauliflora*—a bubblegum coral species for which protocols resulting in high survival remain elusive.

Section I. Update to Boch et al. 2019 Deep-sea Coral Translocation Study

Coral fragments attached using cement (coral pot v.2) continued to exhibit variable survival over time for some of the taxa studied. Both in Boch et al. 2019 and here, we define a “healthy coral fragment” or a colony with natural tissue color and polyps that are turgid and erect. Figure 2A shows an example of an apparently healthy *Corallium* sp. fragment with extended polyps 133 days after translocation. All translocated *Corallium* sp. fragments (n=5) survived ca. one year but survival fell to ~60 % during the second year. Of the initial five fragments, two fragments had broken at the base of attachment and were laying on nearby substrate, with some polyps remaining alive. It is possible that remotely operated vehicle (ROV) operations, interaction with other organisms such as crabs, or insufficient fixing with the cement patcher may have caused these fragments to break or detach from the coral pots, but direct observations were beyond the scope of this study. The corals *S. kofoidi* and *Lillipathes* sp. continued to have high stable survivorship using coral pot v.2 (Figs. 2B, 2C). Survival rates for these corals ranged from ~80-100% over 2 years, with some surviving up to 3 years. After ca. 3 years, survival of translocated *Keratoisis* sp. fragments remained at ~22-25% among both treatments. Survival of fragments held overnight in aquaria aboard a research vessel prior to translocation decreased to 0% for *I. tentaculum* (Figs. 2E, 2F). For *P. arborea* and *S. cauliflora*, the longest observed survival was 338 and 564 days respectively. However, no translocated fragments from these species survived beyond 2 years and methods that improve the long-term survivorship for bubblegum corals will need to be developed (Figs. 2G, 2H).

In 2018, we conducted an additional pilot study at Sur Ridge using three different attachment strategies for *P. arborea*, in hopes of avoiding the tissue degradation, structural vulnerability, or possibly fragment predation that seemed to be occurring along the base of translocated bubblegum coral fragments. To test “protection” against possible negative effects of fast setting cement patcher used to attach fragments, we wrapped the base of fragments with a plastic sheet (Saran™ wrap) and a zip tie was then used to keep the wrap in place at the base of the fragment. To test for structural support at the base of the fragment (and protection against predators at the base of the fragment), we placed the base of fragments inside ¾" diameter Tygon™ tubing, fixed the tubing with zip ties, and then fixed this fragment assembly in the PVC module using fast setting cement patcher. In a third version, we tested for both “protection” against the cement patcher and structural support for the base of the fragment by using the plastic wrap, Tygon® tubing, and zip tie combination. We deployed n=18 total units at Sur Ridge on the same day as collection. We revisited these translocated corals after one year and found that no individuals survived (Table 1).

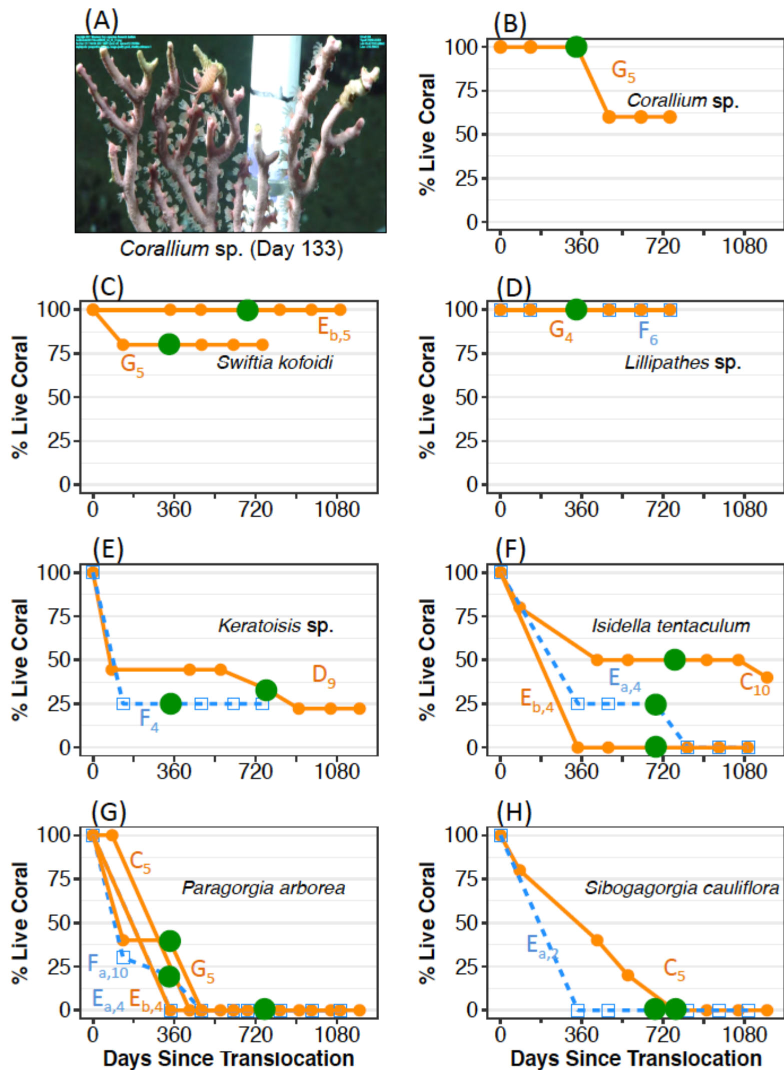


Figure 2. Update to Figure 3 in Boch et al. (2019) by coral species and transport treatment (Sur Ridge, California). Large, green, solid circles represent data up to Boch et al. (2019) publication. (A) An example image of *Corallium* sp. fragment with conspicuous polyp extension at 133 days post translocation. (B) Percent live *Corallium* sp. fragments over time. (C) Percent live *Swiftia kofoidi* fragments over time. (D) Percent live *Lillipathes* sp. fragments over time. (E) Percent live *Keratoisis* sp. fragments over time. (F) Percent live *Isidella tentaculum* fragments over time. (G) Percent live *Paragorgia arborea* fragments over time. (H) Percent live *Sibogagorgia cauliflora* fragments over time. For panels B-H, 0 days = the initial time when coral pots were translocated at depth. Open blue squares represent percent live coral fragments that were exposed to overnight transport in shipboard aquaria; small, solid, orange circles represent survivorship for fragments that were translocated on the same day of collection. Survivorship of coral cohorts is indicated by the letters next to each line, with the initial number of fragments at each deployment expressed as subscripts. Note: all data represent results from deployment using the cement attachment method (coral pot v.2). Photo: MBARI

Table 1. In situ (Sur Ridge, CA) pilot study results for *Paragorgia arborea* translocation using three different fragment attachment strategies.

Date samples collected	Date revisited	Number of fragments deployed	Type fragment attachment	Results
2018-07-15	2019-08-27	6	Wrap + zip tie	No survival
2018-07-15	2019-08-27	6	Tygon tubing + zip tie	No survival
2018-07-15	2019-08-27	6	Wrap + Tygon tubing + zip tie	No survival

Section II. Preparation and Fabrication of Translocation Modules (Coral Pots) for Deep-Sea Coral Fragments

Prior to coral collection, translocation modules should be fabricated to reduce the time required to attach the coral fragments for translocation and deployment. The step-by-step fabrication of the coral pot v.2 used in the Boch et al. (2019) study is illustrated in Fig. 3, and a full list of the materials used is available in Appendix Table 1. It is highly recommended that those using this guide as a reference read through all the steps in both sections prior to cruise departure. In addition, it is recommended that investigators communicate with ROV pilots about the specifications of the coral pot and how the pots may be handled at deployment. The coral pot described here was designed to facilitate ROV operations with the coral pots during deployment, to optimize the number of units that can be placed in the “biobox” of the ROV *Doc Ricketts*, and to minimize the contact that fragments may have with other units—i.e., coral fragments were fixed in the center receptacle.

1. Gather all the necessary materials to build the number of coral pots needed (Fig. 3A, see Appendix Table 1 for full list of materials needed to fabricate one unit).

2. Cut the 1.5" diameter PVC pipe into 3" long pieces (Fig. 3B). These pieces will function as the receptacle for the coral fragments.

3. Cut the ¾" diameter PVC pipe into 2" long pieces (Fig. 3B). These pieces will function as connectors for PVC elbows of the ROV handle attachment to the end cap base.

4. Cut the ¾" diameter PVC pipe into 7" long pieces (Fig. 3B). These pieces will function as connectors for the ROV handle attachment, but the length of these pieces will also depend on the height of the ROV “biobox” or the height of the containers used for deploying the coral pots. **Note:** The length of this piece is where adjustments can be made to meet the requirements of ROV “biobox” or container dimensions.

5. Additional preparation of the coral pot base (Figs. 3B, 3C). Drill ¼" holes into all of the 4" PVC end caps, 1.5" diameter x 3" long PVC pipe pieces, and ¾" x 2" long pieces. Holes should be about ½" to 1" from the bottom. Holes should be drilled so that the PVC pipe pieces and the end cap line up to allow the hex bolt to be pushed through.

6. Build the pot base (Fig. 3C). Connect the end cap and the 2 PVC pipe pieces by threading/pushing the ¼" hex bolt through the pre-drilled holes. Lock the hex bolt in place with a ¼" nut, but only hand tighten. Over-tightening may cause cracks in the end cap.

7. Build the pot handle (Fig. 3D). Use the PVC primer and prime the inside of the T-adaptors, the ends of the 2" and 7" long PVC pieces, and the insides of the elbows. Use PVC cement to glue the T-adaptor to the 7" long PVC piece, ¾" PVC elbow, 2" long PVC piece, and another ¾" PVC elbow as shown in Fig. 3d. Set this completed component aside and let the glue set for at least 15 minutes.

8. Attach the handle to the pot base (Fig. 3E). Once the glue in the handle is set, prime the ¾" x 2" long PVC piece inside the pot base with PVC primer and the inside of the elbow piece in the pot handle. Glue the two pieces together using PVC cement and set aside for at least 15 minutes to set.

9. Complete the coral pot and label each unit (Fig. 3F). Fill the 4" diameter PVC end cap at the base and the center 3" long PVC center receptacle with fast setting cement patcher (Sakrete™). Fill up to the rim of the 4" PVC end cap. The remaining space in the center PVC will be used in the coral fragment attachment part of the process (Section III). **Note:** The coral pot used in the Boch et al. (2019) study weighed approximately 1.2 kg in dry weight without coral fragments.

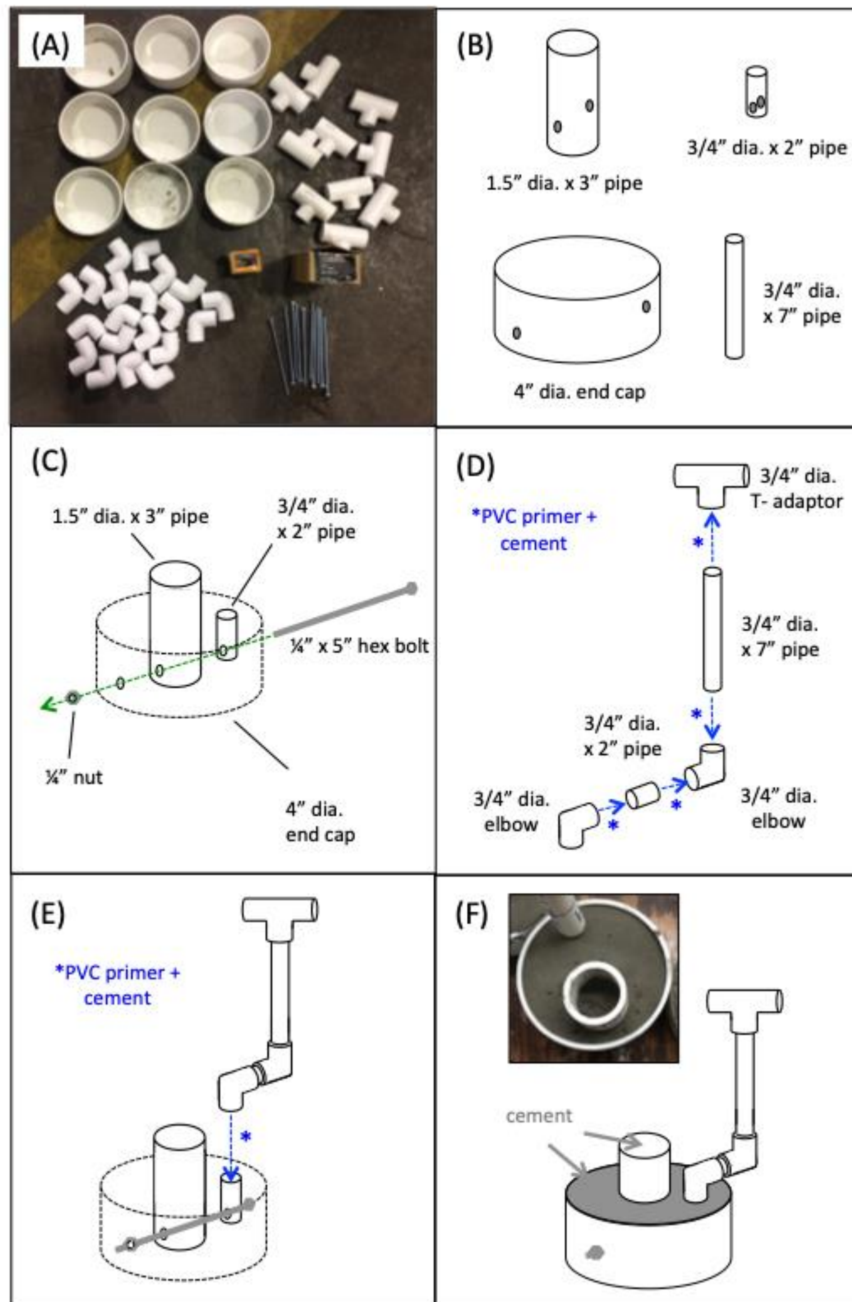


Figure 3. Fabrication of deep-sea coral transport module (coral pot v.2 from Boch et al. 2019). (A) Basic materials needed to construct coral pots. Not pictured are 1.5" diameter x 3" long PVC pipe pieces, ¾" diameter x 7" long PVC pipe pieces, ¾" diameter x 2" long PVC pipe pieces, PVC primer and PVC cement, and the fast setting cement patcher. (B) Base components of the pot are prepared with pre-drilled holes. (C) Base of the pot. (D) Pot handle. (E) Coral pot base is connected to the pot handle. (F) Final coral pot build prior to coral fragment processing. Inset shows an example of the cement in the pot base and center receptacle. Photos and images: C. Boch/MBARI

Section III. Attachment of Coral Fragments to Translocation Modules

Before deep-sea corals are collected and transported to the surface, prepare a working area for coral fragment attachment to the previously prepared coral pots (Fig. 4A). The steps described in this section can then be used as a guide to process and attach fragments of *Corallium* sp., *Lillipathes* sp., *Swiftia kofoidi, Keratoisis* sp., *Isidella tentaculum, Paragorgia arborea,* and *Sibogagorgia cauliflora* to the coral pots. Coral fragments should be attached by hand in the "biobox" containing seawater brought up to the surface from depth. In Boch et al. (2019), seawater temperatures in the "biobox" were approximately 5 °C. This temperature level may be ideal for deep-sea corals, but prolonged exposure to these temperatures may be damaging to human hands. Those involved in this part of the process should take caution and rotate this particular role. Brooke and Young (2009) exposed *Lophelia pertusa* coral fragments to air during the fragment attachment process and concluded air exposure was likely not detrimental for those corals. In Boch et al. (2019), most coral handling activities were performed in seawater to minimize possible stress on corals exposed to air, with the exception of the first two deployments. Those initial transplants were placed loosely, without cement, and exposed to air; this treatment could have been the main cause of 0% survival. We also recommend that a person be designated to note the time, the unique identification of each coral pot, and the type and condition of fragments used for subsequent analysis. Photographing each fragment as it is fixed in the center receptacle also ensures a clear record of when and how long each fragment was handled and processed. Finally, we recommend that a depth and temperature logger be placed in the ROV "biobox" throughout coral collection, processing, and deployment to determine the environmental variability experienced by the corals throughout the process.

1. Prepare the coral fragments (Fig. 4B). Once at the surface, cut the source colonies into smaller fragments using stainless steel scissors. Coral fragments should be cut to similar lengths depending on the type of study but should be shorter in total length than the height of the coral pot and the height of the "biobox". The base portion of the fragment (approximately 1") will be designated as the "anchor" and must be considered when determining the final length desired. Side branches near the base of each fragment may also need some trimming to facilitate placement in the receptacle depending on coral taxon.

2. Prepare the coral pot receptacle for fragment attachment (Fig. 4B). As the donor corals are being processed, place coral pots in the deployment "biobox," which should contain cold seawater brought from depth with the donor coral. Mix cement patcher and place enough in the center receptacle to hold the fragment in place. Because this step is implemented in the

seawater, some of the cement patcher will dissolve and cause sedimentation. The sediment will eventually settle to the bottom. The consistency of the cement patcher should be semi-solid—i.e., not too soft or too set.

3. Fix the coral fragment in the center receptacle (Fig. 4C). Quickly add the coral fragment using needle nose pliers as forceps to insert the coral fragment approximately 1" (~2 cm) into the center receptacle with the fresh cement patcher. Extra cement may be required to fix the fragment in place.

4. Repeat steps 1-3 as necessary for the number of units planned and add ice packs to maintain the temperature of the seawater if needed (Fig. 4D). We highly recommend keeping the corals submerged in seawater throughout the process to limit any additional stress that may be caused by contact with surface air conditions.

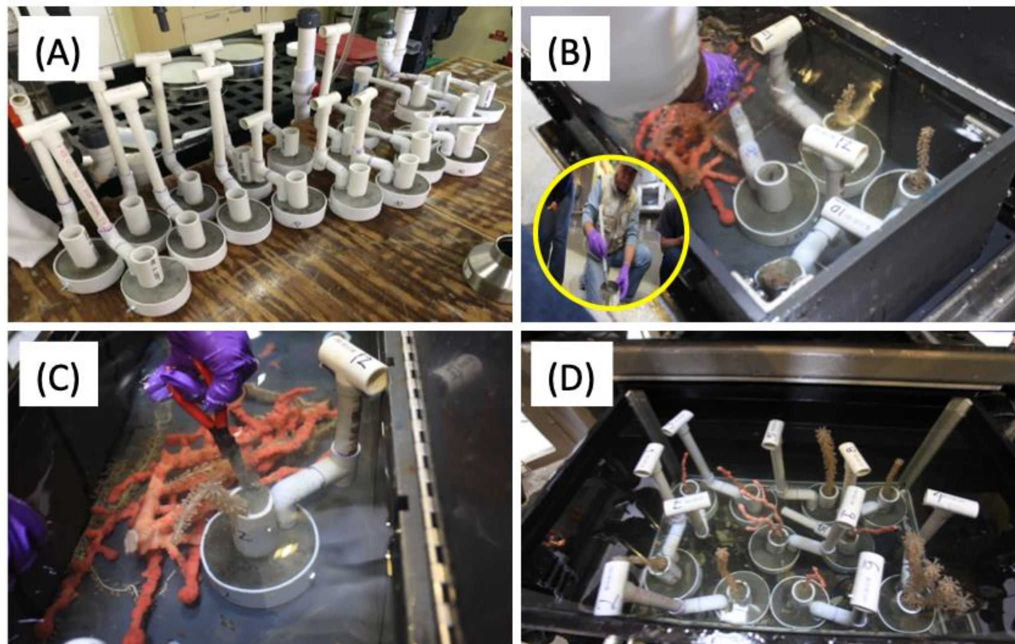


Figure 4. Attaching deep-sea coral fragments into coral pots. (A) Coral pots are prepared prior to coral fragment attachment activities. (B) A coral fragment is cut from a donor colony with stainless steel scissors and fast setting cement patcher is placed into the center 1.5" diameter PVC. Inset photo shows a person dedicated to making fresh batches of fast setting cement during the fragment attachment process. (C) The cut fragment is carefully placed into the center of the 1.5" PVC using straight nose or bent nose pliers, and the fast setting cement patcher is molded around the fragment. (D) Repeat these steps for rest of the coral fragments. Photos: C. King/NOAA and C.Boch/MBARI

Section IV. Insights from the Monterey Bay Aquarium *Sibogagorgia cauliflora* Animal Husbandry Efforts

Background. The Monterey Bay Aquarium (MBA) deep-sea husbandry program focuses primarily on creating a living display reflecting the deep-sea coral and sponge community found near the coast of Monterey Bay, CA for public outreach and education. We are currently developing best-care practices for deep-sea organisms in captivity, and here, initial practical approaches and observations working with *Sibogagorgia cauliflora* are described to further inform future efforts working with bubblegum corals. Similar to the insights gained from the Boch et al. (2019) in situ study, developing a captive program for *S. cauliflora* has been particularly challenging due to a lack of protocols regarding successful mounting strategy, dietary needs, and general environmental parameters needed for long-term survivorship of translocated samples. Details of our initial efforts and observations are described below.

General collection, transport, and set up of *Sibogagorgia cauliflora* samples in the MBA deep-sea animal husbandry program. Specimens of *Sibogagorgia cauliflora* were collected on August 1, 2017 (Sample A, 990 m in depth), April 9, 2018 (Sample B, 993 m), and November 1, 2018 (Sample C, 578 m) using the ROV *Ventana* and the R/V *Rachel Carson* in collaboration with the Monterey Bay Aquarium Research Institute. Collected specimens were placed in the "biobox" drawer of the ROV, brought to the surface, and placed by gloved hand into a large 38"x16"x16" cooler full of 43°F/6.1°C seawater. Efforts to not introduce the corals to air were considered, but we focused on quick transference—with brief exposure to air (seconds)—due to the variable size and shape of each sample. Additionally, coral samples were exposed to natural light, as our main priority was to quickly re-submerge the specimens from the "biobox" to the transportation container. Transportation of deep-sea coral specimens from Moss Landing Harbor to the MBA facilities took approximately 35 minutes after return to the harbor.

Once at the aquarium, coral samples were placed in a 350 gal (1325 L), flow through "deep-sea coral community" sump that also contained other deep-sea corals and anemones. MBA's seawater system circulates seawater pumped in from the nearshore coast (18 m in depth), chills the seawater to approximately 39°F/3.9°C with approximately 1.5 gal/min (5.7 L/min) turnover, in a room illuminated by a single fluorescent fixture dimmed with a red gel filter. The sump is also plumbed with two 0.5 horsepower (HP) pool pumps, one of which delivers seawater to the heat exchanger loop, while the other recirculates the seawater throughout the room; the position of each pump was visually adjusted to help create a turbulent reservoir with indirect flow. There are currently no efforts to reduce dissolved oxygen levels in this system to that of the collection site, but this will likely be examined in the future.

Initial observations of *Sibogagorgia cauliflora* samples in the MBA deep-sea animal husbandry program. In the first effort at keeping *Sibogagorgia cauliflora* in an aquarium system, a whole colony (Table 2, Sample A) was placed in the sump/aquarium tank along with the natural rock to which it was attached. Over the two years since collection, this coral has shown distress in the form of mucus secretion, hydroid overgrowth at the base, and base tissue degradation, yet survived a major chiller malfunction. We observed some of the polyps extended 40% of the time in the first six months, but did not observe polyps feeding at any point in time. At the last point of census (862 days since collection), tissue color around the fan (i.e., branching areas of the gorgonian) remained relatively intact with some base tissue degradation and variability in polyp tissue coloration and polyp extension.

A second sample of a *S. cauliflora* fragment (Table 2, Sample B) was hung upside down by inserting a zip tie ca. 2-3" into the base of the spongy skeleton and secured to a plastic rod with ethyl cyanoacrylate gel. Hanging the fragment presumably allowed it to sway at the mercy of the flow instead of withstanding against it, therefore reducing the need for rigidity and triggering possible tissue breakdown. Overall, Sample B exhibited signs of both tissue degradation and regrowth during the 611 days in the aquarium system. Increased flow seemed to encourage tissue regrowth on the main body of Sample B, supporting speculation that increased flow could encourage tissue regrowth, however this method still has not shown any promise in regrowth of the colony base at rod insertion.

For the third *S. cauliflora* sample (Table 2, Sample C), a smaller (~20 cm) colony was left attached to the natural rock on which it was collected. To examine other attachment effects, the base of Sample C was trimmed of dead tissue and bored out to fit and secure both plastic molly bolts and rods with ethyl cyanoacrylate gel in order to mount the coral on a steady surface. This method was applied based on mounting strategies used on tropical gorgonian species such as *Plexaura* sp. (Lasker 1990), where tissue may be mechanically removed at the base, allowing gorgonian tissue to regrow around the glue in high flow conditions. External tissue of *Sibogagorgia*, however, did not separate from the skeleton cleanly enough, and contact with anything seemed to only further encourage tissue degradation. The insertion approach therefore seemed less invasive by keeping external flesh intact while providing structure enough to support a heavy fan in strong enough flow.

It was unclear if insertion of rods into the fragment base caused further withering; while it seems obvious that coring out tissue, however minimal, might stress the coral, observations of Samples A and C, both naturally fixed to rocks, exhibited the same characteristic tissue degradation at the base. Since this coral depends entirely on filter feeding for nutrient uptake, it could be that nutrient-deficient corals simply start to erode at the base first. While less invasive methods of mounting will still be explored, rod insertion will not yet be ruled out as a potential mounting method, and

Sample C will continue to be observed to assess polyp extension and feeding behavior.

The initial coral diet consisted of *Artemia* nauplii blended with liquid food items from Reed Mariculture's Shellfish Diet 1800® algae paste, Reef Nutrition® Roti-Feast®, and Reef Nutrition® Oyster-Feast® in an effort to offer a variety of common coral food types. Since polyp feeding was not observed with this blend, we waited for periods of polyp extension and offered isolated food items from the *Artemia* blend to be gently basted over any open polyps, under white light, to determine particle choice. Polyp behavior was categorized as uninterested, catching and releasing, and catching and consuming. Polyps clearly favored small particle size. Larger *Artemia* nauplii were entirely rejected, as were other large size particles, such as wild 1-2 mm calanoid copepods and 1-2 mm cultured cylopeze copepods. Other small particle diets, such as the popular gorgonian food, Golden Pearls (100-200 micron), were observed to be consumed. After Sample B was shown to favor small particles, the liquid diet blend was diluted and broadcast by a peristaltic dosing pump over 12-hour periods.

Daily bastings seemed necessary, even with strong flow. Mucus sheets formed during periods of polyp dormancy. After brushing the tip of the fragment to break the film, basting it off resulted in polyp exposure shortly thereafter. Hydroid growth was gently removed with a soft brush, as it was speculated that these epifauna might overrun a coral, especially in tanks with constant feeding regimens. Polyp extension was variable over coral branches, where only 1-2 polyps would extend over time, suggesting a differential acclimation period to a new pattern of flow. It is not yet clear if this species requires constant flow, pulsing flow, or any periods of rest.

Sample C went through similar husbandry care as Sample B and also showed signs of tissue degradation after translocation to the aquarium system. Over a period of 405 days, we observed tissue degradation but also polyp extension in some parts of the coral. This coral sample had initially fallen over in the sump and degradation of tissue was especially apparent on the side of the coral touching the tank. Reattaching the coral sample vertically to a plastic molly rod seemed to restore some of the polyps that may have been impacted from falling over.

Overall, the three samples of *S. cauliflora* showed signs of stress after translocation to the aquarium system in the form of limited polyp extension, tissue degradation, and color loss in the tissue. Polyps were rarely observed to feed, which will be a major factor to address for deep-sea organisms kept in captivity.

Table 2. General summary of *Sibogagorgia cauliflora* husbandry efforts and observations at the Monterey Bay Aquarium (Monterey Bay, CA). Three samples of *P. arborea* corals were collected (from Sur Ridge, CA), and are hereafter referred to as A, B, and C. *Artemia* blend consisted of 2 L of *Artemia* nauplii with 50 mL each of Reed Mariculture's Shellfish Diet 1800® algae paste, Reef Nutrition® Roti-Feast®, and Reef Nutrition® Oyster-Feast® with fortified blended krill (once a day by pouring into the tank). Golden pearls blend consisted of 10 mL Shellfish Diet 1800® algal paste and 250 mL each of Roti-Feast® and Oyster-Feast® emulsified into 50 mL of 100-200 micron Golden Pearl diet (basted indirectly over the polyps). Golden pearl bastings were supplemented with the liquid food blend diluted to 30 L of seawater and fed using a peristaltic pump over a 12-hour period. This also allowed broadcast feeding to other species in the 350-gallon sump. Table continued on next page.

Sample	Date of Observation	Type of Attachment	Flow Control	Husbandry	General Coral Condition and Observations
A	8/1/2017-10/27/2018	Coral left on natural rock	Rio 180 powerhead	Moved into the sump to consolidate after months of not being open; directly fed *Artemia* blend; basted and brushed	Mucus and hydroids observed on and around coral. Polyps extended ~ 40% of the time first half of the year but no observations of feeding.
	10/28/2018-10/18/2019		Hayward 0.5 HP recirc. pump	Brush and baste coral, introduce more flow; directly fed *Artemia* blend, then dosed indirectly using flow	Thinning of branches, mucus membrane coated with bacteria and sediment, hydroids growing from base rock onto withered base. No evidence of polyp extension or feeding.
	10/19/2019-11/30/2019		Hayward 0.5 HP recirc. pump + Maxspect XF350 gyre set	Increased flow, cleaned with brush and baster, and watched polyps for feeding opportunities with Golden Pearls blend.	Base was still degraded; polyps on entire coral showing slight bumps instead of smooth and withered; no observations of feeding.
B	4/09/2018-8/09/2019	Coral fragment attached using zip tie and placed hanging upside down	Hayward 0.5 HP recirc. pump	Secured coral to rack surface to allow stronger flow after base snapped at zip tie	Seemingly well under red light; under white light inspection, major surface tissue loss observed; no evidence of polyp exposure or feeding.
	8/09/2019-8/22/2019	Molly bolt, vertical surface	Hayward 0.5 HP recirc. pump	Secured coral to rack surface to allow stronger flow after base snapped at zip tie.	Cleaned with trimmed base. No observation of polyp extension.

Sample	Date of Observation	Type of Attachment	Flow Control	Husbandry	General Coral Condition and Observations
	8/23/2019-9/13-2019	Molly bolt, vertical surface	Hayward 0.5 HP recirc. pump	Basted, brushed, fed *Artemia* blend	Cleaned with trimmed base. No evidence of polyp exposure or feeding.
	8/23/2019-9/13/2019	Plastic rod; vertical surface	Hayward 0.5 HP recirc. pump + OW-40 Jebao wavemaker	Basted with Golden Pearl blend; move to horizontal surface and secured with thinner plastic rod	Underside of fragment stripped of pink tissue and polyps, snapped off at molly bolt; some polyp extension near top branch tips.
	10/19/2019-11/30/2019	Plastic rod, horizontal surface	Hayward 0.5 HP recirc. pump + OW-40 Jebao wavemaker	Aggressively brushed mucus, introduced stronger flow; increased Golden Pearl basting	Side previously considered underside regrew, polyps started to return, base continued to wither; polyps consistently extended, except for one branch with mucus.
	10/19/2019-11/30/2019	Plastic rod, horizontal surface	Hayward 0.5 HP recirc pump + Maxspect XF350 gyre set	Continued flow trial, potentially reposition between two gyres	Pink tissue seemed to be restoring with some polyps returning on one side and some not extended, possibly acclimating to new flow.
C	11/01/2018-8/09/2019	Coral left on natural rock; rock mounted to rack vertically	Hayward 0.5 HP recirc. pump	Basted often	Found fallen and degraded on side resting on tank with some base tissue missing; no evidence of polyp exposure or feeding.
	8/22/2019-9/13/2019		Hayward 0.5 HP recirc. pump + OW-40 Jebao wavemaker	Basted to clean and feed Golden Pearls blend	Area previously resting on side degraded and exposing white skeleton, base still slightly withered; polyps extended on healthy tissue.
	9/14/2019-10/19/2019		Hayward 0.5 HP recirc. pump + OW-40 Jebao wavemaker	Continued to clean, expose to flow, baste, and feed Golden Pearls blend	Previously degraded area of coral sloughed off film to reveal new pink tissue; base was still slightly withered; polyps extended regularly.

DISCUSSION

As human activities have increasingly broad and profound effects in the deep sea, restoration of impacted deep-sea populations and husbandry of deep-sea organisms in captivity are new frontiers for ocean science and resource management. The effects of trawling on deep-sea coral and sponge communities were perhaps the first deep-sea ecosystem impacts to be highlighted (Koslow et al. 2000; Van Dover 2014; Van Dover et al. 2014; Amoroso et al. 2018). We now face continuing fishing impacts, as well as host of new threats ranging from deep-sea mining to climate-linked changes in ocean conditions. The 2010 Deepwater Horizon oil spill and its enormous scope refocused our attention on anthropogenic impacts in the deep sea. Constraining further impacts using networks of marine protected areas, a successful approach in shallow marine environments (McCook et al. 2010), shows promise, but has only been applied sparingly in the deep sea (Edgar et al. 2014). Studies of deep-sea restoration methods remain limited, but the initial insights show some promise and provide a foundation to build on. The study by Boch et al. (2019) is one of the first to explore methods to actively promote the recovery of impacted deep-sea coral populations by examining the performance of multiple coral taxa. Here, we have described the fabrication of coral pots and the process of fragment attachment in more detail, in support of future efforts toward deep-sea coral restoration. The insights gained from animal husbandry efforts by a public aquarium also provided additional insights, revealing similar challenges that must be overcome to successfully translocate and keep deep-sea corals alive and healthy.

The pilot study using three different attachment strategies beyond those reported in Boch et al. (2019) for *Paragorgia arborea* indicate that successful translocation of *P. arborea* fragments remains elusive for long-term in situ efforts. Efforts to keep a similar bubblegum coral taxon (*Sibogagorgia arborea*) in a captive setting also remain challenging. However, insights from the in situ and captive efforts have independently converged on at least three initial factors that need to be overcome in order to successfully enhance the survivorship of some species of bubblegum corals. One, reducing tissue degradation of both *P. arborea* and *S. cauliflora* when mounted on substrates using artificial means are critical. Feeding rates or diet composition are likely essential for the health of the coral in both the natural environment and aquarium systems, and flow regimes will likely influence the delivery of available food. Furthermore, complete life history of bubblegum corals and especially reproduction requirements remains a major gap in deep-sea restoration efforts and captive husbandry programs. While there are many practical protocols that need to be further evaluated, these three factors will likely help lay the foundation for future efforts.

The cost of enhancing the restoration and recovery of deep-sea coral communities after anthropogenic disturbances will remain uncertain until all components of

ecosystem services and the scale of active mitigation strategies can be explored. In our study, the use of the R/V *Western Flyer* and the ROV *Doc Ricketts* cost approximately $30,000 per day, with the study site within 4 hours from port. However, the cost of establishing viable translocation methods here should also include the costs of mapping deep-sea coral communities and re-visiting mitigated areas so that outcomes of translocation efforts can be assessed in a rigorous manner over time. Additional evaluations will also be needed to examine larger and cost-effective aquarium systems that minimize stress while transporting deep-sea corals over long distances and time periods, which may be necessary to mitigate large scale anthropogenic disturbances such as the Deepwater Horizon oil spill (Deepwater Horizon Natural Resource Damage Assessment Trustees 2016). Despite the need for further development, the relatively high survivorship of deep-sea coral fragments in pots constructed of low-cost materials (~US $20 per pot) is a promising indication that developing active mitigation strategies for deep-sea corals could have merit. We also acknowledge that PVC and cement materials are not ideal as a permanent translocation solution and may not be robust in the long-term, as we observed several coral pots break apart over time. We highly recommend that additional bio-friendly materials, such as biodegradable cardboard, be explored as transport modules.

The long-term survival of translocated corals, as well as their effect on deep-sea coral recovery over decades to centuries, is yet to be determined. Considering the slow growth rates and high longevity of deep-sea corals (Andrews et al. 2002; Andrews et al. 2005; Roark et al. 2005), it is natural to question if coral translocation is likely to accelerate the recovery of coral populations in damaged habitats. Additionally, understanding the impacts of sourcing coral fragments from “healthy” versus “unhealthy” or “dying” donor colonies will be a critical step prior to implementation. Deep-sea donor corals from which we removed branches healed without visible impacts, but these broader questions have not been studied in detail. Despite the gaps in knowledge, we will need to ask what role active mitigation will play in response to past, current, and future changes in the ocean due to increased human activity. Will establishing cost-effective restoration approaches that enhance gamete contact and approaches that generate corals that are more resilient to climate-related changes in ocean conditions better prepare deep-sea ecosystems for the future? Perhaps efforts to propagate other key associated taxa such as sponges that may enhance energy flow and carbon sequestration could help mitigate climate change driven changes in the deep sea (Murray et al. 1994; Cathalot et al. 2015; Kahn et al. 2015). As these and other questions continue to be investigated, developing methods for evaluating the success of restoration will require clear documentation and testing of the approaches used. Overall, the most effective strategies for mitigating damage in deep-sea coral habitats are uncertain, but exploring the potential value of restoration options such as coral translocation and other approaches will help shape our efforts to protect and sustain these valuable and fragile deep-sea resources.

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HP	horsepower
MBA	Monterey Bay Aquarium
MBARI	Monterey Bay Aquarium Research Institute
NOAA	National Oceanic and Atmospheric Administration
PVC	polyvinyl chloride
ROV	Remotely Operated Vehicle
R/V	Research Vessel
sp.	species

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APPENDIX

Table 1. The items listed below were used to fabricate a single coral pot in reference to this technical document (from Boch et al. 2019). Additional items are listed for the coral fragment attachment process. Sakrete™ fast setting patcher is available in different volumes, and a 20 lb. source can be used to fabricate multiple coral pots.

	Item	Number of units
1	¾" diameter schedule 40 PVC pipe	2 x 2"
2	¾" diameter schedule 40 PVC pipe	1 x 7"
3	1.5" diameter schedule 40 PVC pipe	1 x 3"
4	4" diameter schedule 40 PVC end cap	1
5	¾" diameter schedule 40 PVC pipe elbow	2
6	¾" diameter schedule 40 PVC T-adaptor	1
7	2 - ½ "capacity PVC cutter or band saw	1
8	¼" drill bit	1
9	¼" hex bolt	1
10	¼" nut	1
11	PVC primer	1
12	PVC cement	1
13	Sakrete™ fast setting patcher	1 x 20 lb
14	Stainless steel scissors	1
15	Bent nose pliers	1
16	Ice packs	20
17	Thermometer	1

America's Underwater Treasures