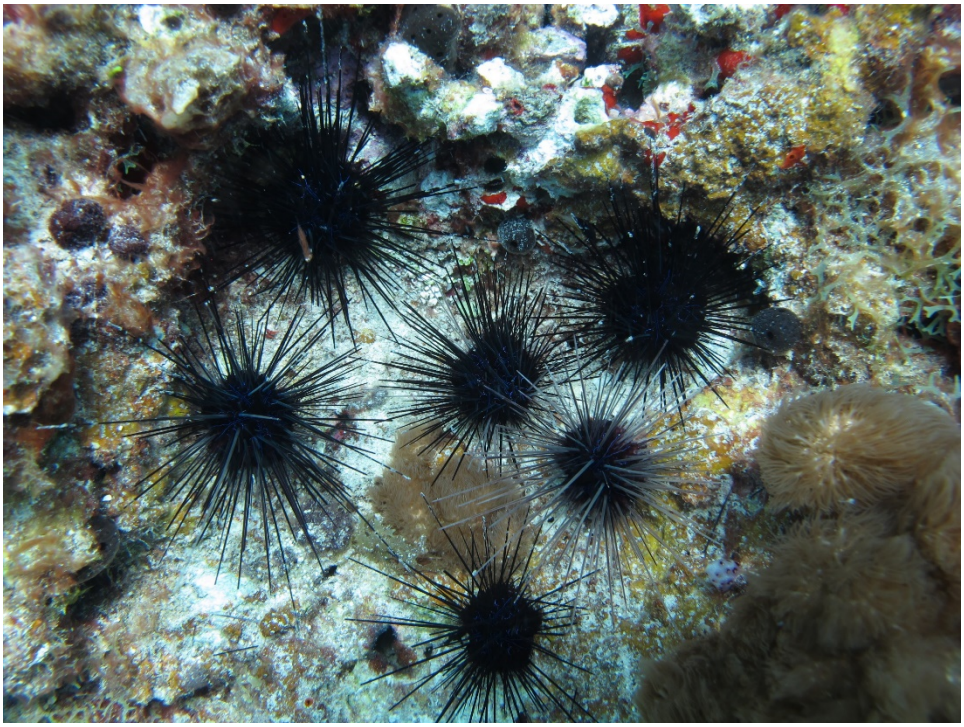
The control of algal abundance on coral reef habitats in Puerto Rico through the restocking of *Diadema antillarum*



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Executive Summary

In Puerto Rico, a more recent and potential threat to the coral reefs is the increasing abundance of a red encrusting algae, *Ramicrusta* sp. *Ramicrusta* is an encrusting red alga from the Peyssonneliaceae family (Rhodophyta). *Ramicrusta* was observed at 70% of the monitoring sites (42 sites) in the 2017-18 DNER/NOAA Coral Reef Monitoring surveys. Moreover, *Ramicrusta* sp. was the main benthic substrate observed on shallow-water coral reefs at a majority of the reefs surveyed in Culebra Island and Fajardo. *Diadema antillarum*, a keystone herbivore, has been witnessed eating *Ramicrusta* (Williams pers comm, Ruiz 2015, Williams 2017) and in the field we observed a noticeably lower cover of *Ramicrusta* in areas where *D. antillarum* were present. The goal of this project was to increase herbivory rates on two coral reefs in Fajardo and decrease algal abundance, specifically *Ramicrusta* sp. In addition, it is also expected, that effective recruitment rates will increase as an indirect consequence of *D. antillarum* herbivory effect.

A total of 3, 179 *D. antillarum* settlers were collected during the summer of 2017. They were brought back to the laboratory at the University of Puerto Rico and grown to a young adult size (test diameter between 3cm to 4cm). The survivorship of the individuals grown in laboratory was impacted by the passing of Hurricane Irma and Maria. *D. antillarum* settlers had to be placed in cages under the dock at the university because there was no electricity for three months. On January 4, 2018, 505 sea urchins were removed from the cages and placed back in the raceways outside (37% survivorship).

On August 22, 2018, at 1:00 PM, 480 young adults (3-4cm) were transferred to Cayo Diablo (240 in total) and Los Lobos (240 in total). Twenty-five urchins were placed in six corrals that were installed at each reef. Tops were installed on three of the corrals to fully enclose (hereafter closed) the area, and three corrals did not have tops. Most of the *D. antillarum* escaped

the corrals a day after the restocking. Overall, the retention of individuals in corrals was greater in the closed corrals than in the open corrals. After the first month, an average of 44% and 52% or the restocked individuals were recorded in fully closed corrals in Los Lobos and Cayo Diablo, respectively. The majority of the corrals were completely destroyed and open by the second month, therefore the sea urchins were no longer confined. Therefore, the numbers of *D. antillarum* located in the corrals decreased even more so by the second month of monitoring.

The benthic composition did not vary at the control stations through the monitoring period. Therefore, it can be assumed that the changes observed in the benthic cover inside the corrals, both open and closed, are due to the grazing of restocked *D. antillarum*. One week after the restocking, the benthic substrate was characterized by more turf algae, and less *Ramicrusta*. There was a shift in benthic community structure after one and two months after the restocking (9/22/2018, 10/23/2018). The difference in benthic assemblage after one month was due to the reduction of *Ramicrusta* cover. The abundance of *Ramicrusta* was significantly reduced over the monitoring period (Indices 3-4). The reduction in *Ramicrusta* was slightly more evident at Cayo Diablo than at Los Lobos; where *Ramicrusta* was reduced as much as 63% at Cayo Diablo (Fig. 16) and 61% at Los Lobos (See Indices 5 and 6). After one and two months, the substrate inside the corrals was characterized by more available space, turf algae, for corals and other benthic organisms to settle and colonize. The evidence presented in this study supports that the action of restocking of *Diadema antillarum* may be useful mitigation tool to control *Ramicrusta* and other algal abundance on the coral reefs.

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Introduction

Over the past four decades, coral reefs in the Caribbean have dramatically changed, (Hughes 1994, Wilkinson 2008, Jackson et al. 2014). The abundance of reef-associated organisms, especially corals, has suffered a massive decline due to cumulative factors such as, hurricanes, disease outbreaks, bleaching, pollution, and overfishing (Bythell and Sheppard 1993, Bythell et al. 1993, Littler et al. 1993, Hughes 1994, Kramer et al. 2003). One of the most dramatic shifts in community structure occurred after the massive die-off of *Diadema antillarum*, a keystone herbivore. The 1983-1984 mass mortality of *D. antillarum* occurred throughout the Caribbean basin and was the most extensive and severe die-off ever recorded for a marine invertebrate (Lessios 1995). Before 1983, the presence of this organism was common (13-18 Ind m^{-2}) on coral reefs in the in Puerto Rico (Bauer 1980, Vicente and Goenaga 1984), and played an important role in structuring coral reef communities by controlling algal abundance (Carpenter 1981, Carpenter 1986, Carpenter 1990a, Carpenter 1990b, de Ruyter van Steveninck and Bak 1986, Odgen et al. 1973, Robertson 1987, Sammarco 1982), productivity (Williams 1990) and is one of the principal agents of bioerosion on reefs (Lidz and Hallock 2000, Bak et al. 1984, Scoffin et al. 1980). After the massive die-off, populations were drastically reduced by 95-100% in many Caribbean locations (Lessios 1995), and at the same time, fleshy macroalgal cover increased between 100% and 250% (Phinney et al. 2001). The absence of *D. antillarum* did not only influence the benthic algal productivity of coral reef communities, but it also impinged on the settlement of coral recruits.

Presently, the recovery of *D. antillarum* has been slow and even absent at many locations in the Caribbean (Lessios 2016). In Puerto Rico, there has been a modest recovery in the population of *D. antillarum* (Mercado-Molina et al. 2014), nevertheless, densities are still far below pre-mass mortality numbers (Lessios 2016). It has been proposed that either larval mortality and/or post-

recruitment mortality processes could be the main factors regulating the adult population size of *D. antillarum* (Karlson and Levitan 1990). In Puerto Rico, in particular, the former has been discarded as it has been shown that upstream sources of "settlement-ready" larvae for *D. antillarum* are available (Williams et al. 2010); therefore larval supply and survival do not seem to be inhibiting the recovery of these populations. Consequently, recruitment-limited processes, such as post-settler and/or juvenile mortality may be regulating the population dynamics of *D. antillarum* in Puerto Rico.

It is assumed that, after a disturbance and under the right circumstances, an ecosystem will recover (Nystrom and Folke 2001); however many reefs in the world, and in particular in the Caribbean, have lost their capacity to recover from recurrent disturbances and have undergone long-term phase shifts (Hughes et al. 2003). The most spoken about phase-shift in scientific literature is the coral to fleshy macroalgal shift (Hughes 1994, Shulman and Robertson 1996, McClanahan and Muthiga 1998, Rogers and Miller 2006). Reefs characterized in permanent states of algal dominance usually signifies a loss of resiliency (Hughes et al. 2007) because macroalgal assemblages limit coral settlement, affect sediment deposition, and alter chemical properties close to the benthos (Birrell et al. 2008). In Puerto Rico, a more recent and potential threat to the coral reefs is the increasing abundance of a red encrusting algae, *Ramicrusta* sp. *Ramicrusta* is an encrusting red alga from the Peyssonneliaceae family (Rhodophyta). Currently there are three species of *Ramicrusta* identified in the Caribbean; *textilis*, *bonairensis*, and *monensis* (Ballentine et al. 2016). *Ramicrusta* forms a thin, crustose layer that spreads over the substrate and can grow over the living tissues of other organisms (Pueschel and Saunders 2009, Eckrich and Engel 2013, Ballentine et al. 2011, Ruiz 2015). In Bonaire, Eckrich and Engel 2013) observed the maximum overgrowth rates to range between 0.06 mm d^{-1} and 0.08 mm d^{-1}. The rapid growth of *Ramicrusta*

makes this algae not only a threat to slow growing sessile-benthic organisms but has the potential to reduce the area of suitable substratum for coral settlement. Species of corals that can be negatively impacted by the expansion of *Ramicrust*a include the ESA listed species of *Acropora* and *Orbicella*.

During 2016, *Ramicrusta* cover was high (41-75%) on Fajardo, Culebra Island, and Vieques reefs. By 2017-18 surveys Ramicrusta was observed at 70% of the monitoring sites (42 sites). Moreover, *Ramicrusta* sp. was the main benthic substrate observed on shallow-water coral reefs at a majority of the reefs surveyed in Culebra Island and Fajardo. On the east coast, the abundance of *Ramicrusta* was astoundingly high and dominated the benthic composition at the majority of sites and depths, with the exception at the deeper sites at Culebra and Fajardo. Even more concerning was that *Ramicrusta* was more abundant than coral at 8 out of the 9 sites on the east coast and the cover was more than double that of coral cover at half of these sites. During 2018, we observed *Ramicrusta* overgrowing *Orbicella annularis* and *Acropora cervicornis*, among other corals at reefs in Culebra (see Figure 1).

Diadema antillarum, a keystone herbivore, has been witnessed eating *Ramicrusta* (Williams pers comm, Ruiz 2015, Williams 2017) and in the field we observed a noticeably lower cover of *Ramicrusta* in areas where *D. antillarum* were present. The goal of this project was to increase herbivory rates on two coral reefs in Fajardo and decrease algal abundance, specifically *Ramicrusta* sp. In addition, it is also expected, that effective recruitment rates will increase as an indirect consequence of *D. antillarum* herbivory effect.

Methods

Collection

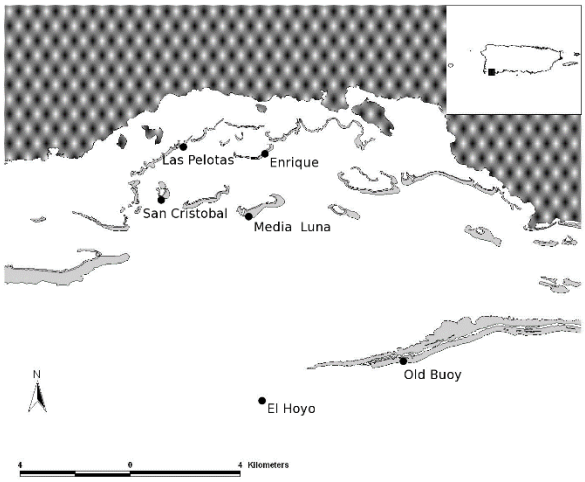


Figure 1 .Settlement sites in La Parguera, Puerto Rico

The settlement of *D. antillarum* is patchy and at times hard to identify and collect. We followed the methodology of Williams et al. (2010, 2011) settlement studies. They concluded that shelf-edge sites at La Parguera, Puerto Rico, Old Buoy and El Hoyo were "hot spots" for *D. antillarum* settlement (max of 1,064 Ind m^{-2}). The supply of *D. antillarum* settlers for this restoration experiment were collected at the Old Buoy (Fig. 1). The depth of the site ranges from 18-21 meters.

The reef substrate at Old Buoy is fairly flat with relatively low coral cover and diversity. Historically, there have been few adult *D. antillarum* recorded at both of these sites (<0.01 Ind m^{-2}). Thirty mooring lines were placed at Old Buoy. On each mooring line, a rope containing 20, 8 × 8 cm pieces of artificial turf (Fig. 2) was attached to the cement blocks and mooring buoy (600 plates in total). The cement blocks were placed in the middle of a sand channel, and not impacting the corals and reef on either side. The buoys were located at 6 m of depth and did not represent any navigational hazard. Plates were positioned in 9.8 meters of depth in the water column. During collections, the rope was detached and placed in a dry bag and a new rope with

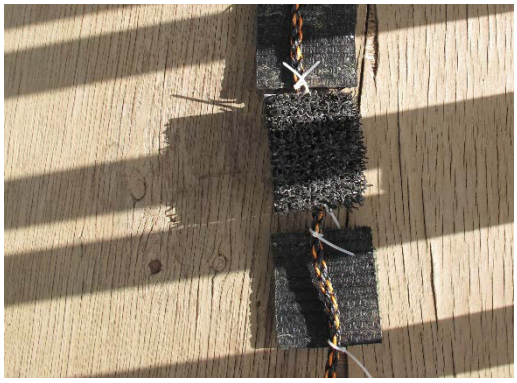


Figure 2 Astroturf or artificial turf used as settlement

clean plates was attached. Settlement plates were replaced each month on twenty lines. On the other ten lines, plates were replaced every two months from June to September 2017 (total of five samplings). Plates were brought back to the laboratory to analyze.

Grow-out

Settlers (Fig. 3) were picked off each settlement plates, counted and transferred to a 45-gallon and 60-gallon tank. Both tanks were connected to a closed-filtering system. The closed system helped reduce sediment and other larvae from entering and settling in the tanks, while maintaining water quality (salinity and temperature). Tanks were cleaned once a week and one-third of the water was replaced with fresh seawater that was filtered and run through a UV light.

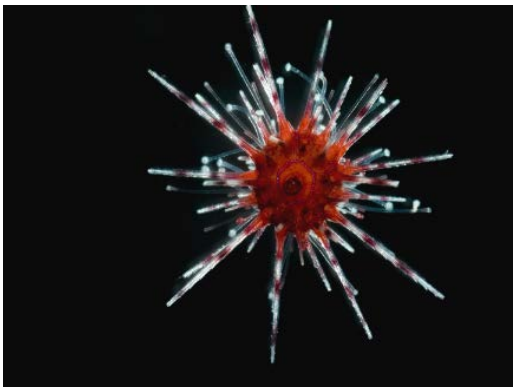


Figure 3 Diadema antillarum settler

Water quality measurements were recorded every week. These measurements included salinity, pH, temperature, nitrate, and ammonium. Settlers were transferred to wet tables or raceways (Fig. 4) once they reached a size of 5 mm in test diameter. Raceways were connected to a semi-closed circulating system. This allowed the water to be recycled through the tanks even if fresh seawater was not being supplied. The system was flushed one a week and fresh saltwater entering the system is filtered through a sock. The sock was replaced and cleaned once a week and raceways were cleaned once every two weeks.

Figure 4 Wet laboratory at the University of Puerto Rico, Mayaguez.

Algae were collected and placed in each raceway every three to four days. Algal species, *Acanthomorpha*, *Chaetomorpha*, *Padina*, *Stypopodium*, and *Dictyota* spp., were collected in the field once a week and kept in a holding tank. *D. antillarum* juveniles were transplanted to the reef once they reached a test size of at least 3-4 cm in test diameter.

Restocking

D. antillarum juveniles were transplanted to the backreef of Cayo Diablo and Los Lobos in Fajardo, Puerto Rico (Figure 5). Six corrals were installed at each reef at 5-7 meters and held into place with rebar. The diameter of each corral was approximately $2m^2$. Corrals were made of galvanized chicken wire with a 1-inch diameter mesh size. The plastic chicken wire was attached to the bottom of the corral to mold to the reef. Corrals were placed in the sand around isolated *O. annularis* colonies. At each reef, tops were installed on three of the corrals to enclose (hereafter closed) the area fully, and three corrals did not have tops. Monitoring took place one day,

one week, one and two-month after the reintroduction. Individuals were counted inside and outside the corrals. Corrals were removed after the two month monitoring.

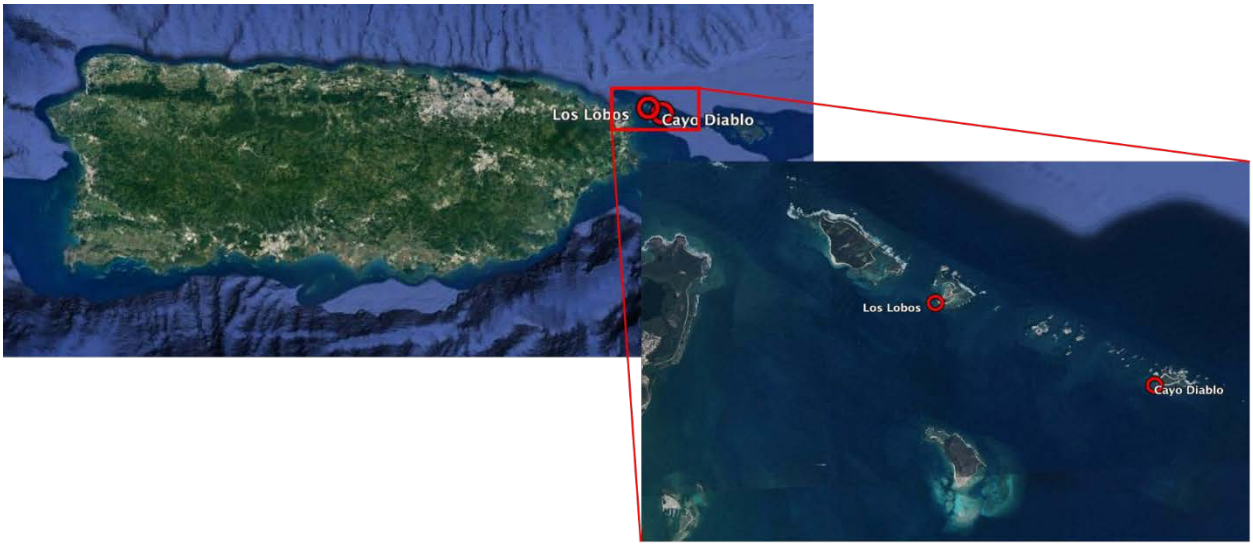
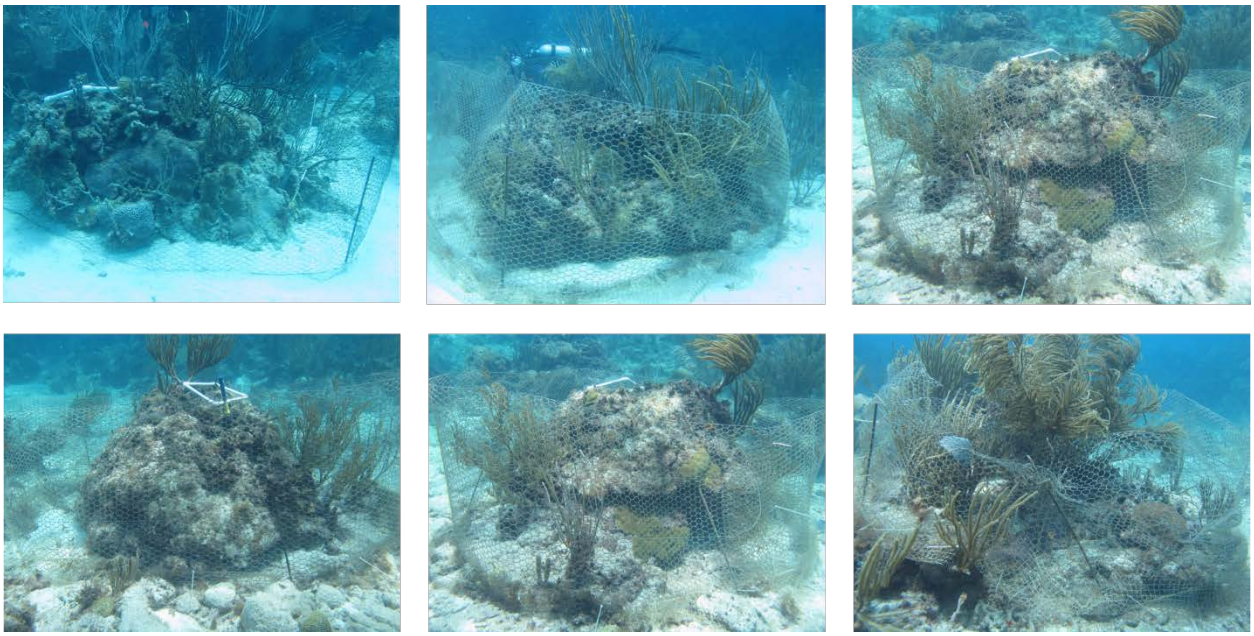


Figure 5 Map of the restoration sites, Cayo Diablo and Los Lobos in Fajardo, Puerto Rico.

Six 25 cm x 25 cm quadrats, with three random and three fixed were placed and photographed inside of each corral to monitor benthic cover through time. Nails will be used to mark the position of fixed quadrats, allowing for the estimation of change. In addition, change in benthic composition was also monitored outside the corrals (control) with three random and fixed quadrats. Percentage cover of algae was discriminated to the lowest possible taxonomic level. The photographs were examined in the laboratory to estimate relative percentage cover of sessile organisms. Observations on percentage cover of other benthic organisms were also recorded. Photographs were not taken at Los Lobos after one month of restocking because of a camera malfunction.



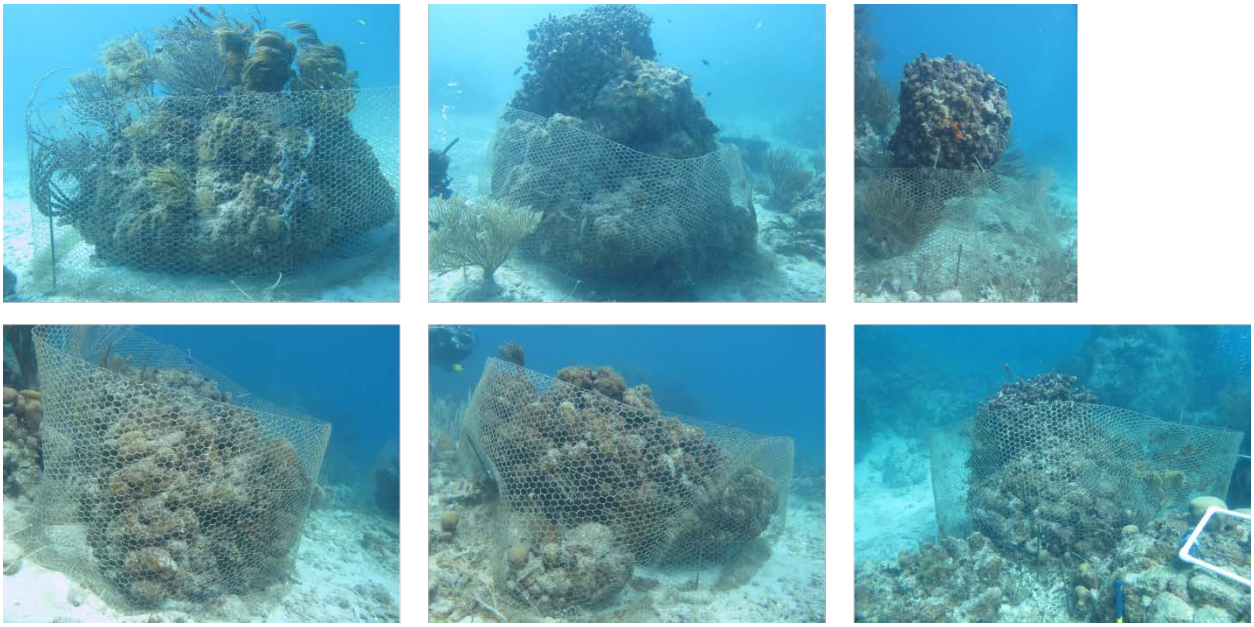


Figure 6 Pictures of the different corrals in Cayo Diablo (top six pictures) and in Los Lobos (bottom six), in Fajardo, Puerto Rico. Corral number is arranged from left to right.

Statistics

Three-way distance Permutational Multivariate Analyses of Variance (PERMANOVA) tests (Anderson 2001) were performed at each site (Cayo Diablo and Los Lobos) to examine corral type (open or closed), quadrat type (fixed and random), and date. The sites were examined separately because the dataset was unbalanced, for example, the benthic composition was not measured one month after restocking at Los Lobos. Corral 6 was not analyzed at each of the sites because all *D. antillarum* escaped after one week of restocking. Each PERMANOVA procedure was based on Bray-Curtis similarity measures. SIMPER test were run to identify the contribution of benthic categories to the overall differences between years with the random and fixed quadrats and at the different sites. A Principal Component Analysis (PCA) plots were produced to visualize differences in benthic assemblages between years.

PERMANOVA analyses were also run to examine the differences in *Ramicrusta* cover between the sites, corral type and dates. Again, separate analyses were tested for random and fixed

quadrats. For the analyses of *Ramicrusta* cover (univariate), the similarity matrix was based on Euclidean distances. Euclidean distance measures for univariate PERMANOVA analyses produce sums-of-squares estimates equivalent to parametric ANOVA (Anderson, 2001), and allow the same methodological framework to be used for all community attributes.

Results and Discussion

Collection

Twenty lines were cut and stolen during the August collection. The lines were cut again in September, but we arrived at the site and were able to retrieve them as they floated away. Therefore, only 200 plates were analyzed during August. The mooring lines were removed during our collection in September, and we did not proceed with October collection because of weather (hurricanes). The total amount of settlers collected from June to September are listed below in the table (Table 1)

Table 1. The total amount of settlers (#) collected at each sites from June to September 2017.

Month	Settlers
June	259
July	1355
August	439
September	1126
Total	**3179**

Grow-out

There were some modifications this year because Hurricane Irma hit the island on September 5, 2018 and Hurricane Maria on September 20, 2018. Electricity was lost after Irma for about week but most of the island was without electricity, especially Isla Magueyes for many months (about three months) after Maria. The university ran the generators for only eight hours a

day. Many sea urchins started perishing in the tanks, possibly due to oxygen constraints and the daily temperature variation, since the water was not flowing throughout the night. On October 12, 2017, I decided to move all sea urchins to cages and hung these cages under the dock. A total of 1,367 sea urchins were placed in two cages (43% survivorship). Cages were made out of PVC tubes and screen. It was challenging to keep potential predators (mantis shrimps, worms, and crabs) outside of cages. On January 4, 2018, 505 sea urchins were removed from the cages and placed back in the raceways outside (37% survivorship).

Restocking

Survivorship

On August 22, 2018, at 1:00 PM, 480 young adults (3-4cm) were transferred to Cayo Diablo (240 in total) and Los Lobos (240 in total). Reintroducing *D. antillarum* during late afternoon hours allows for adaption to reef conditions overnight. Twenty-five *D. antillarum* were placed in each corral (~6 Ind m^{-2}). The rest of the *D. antillarum* were released freely on the reef.

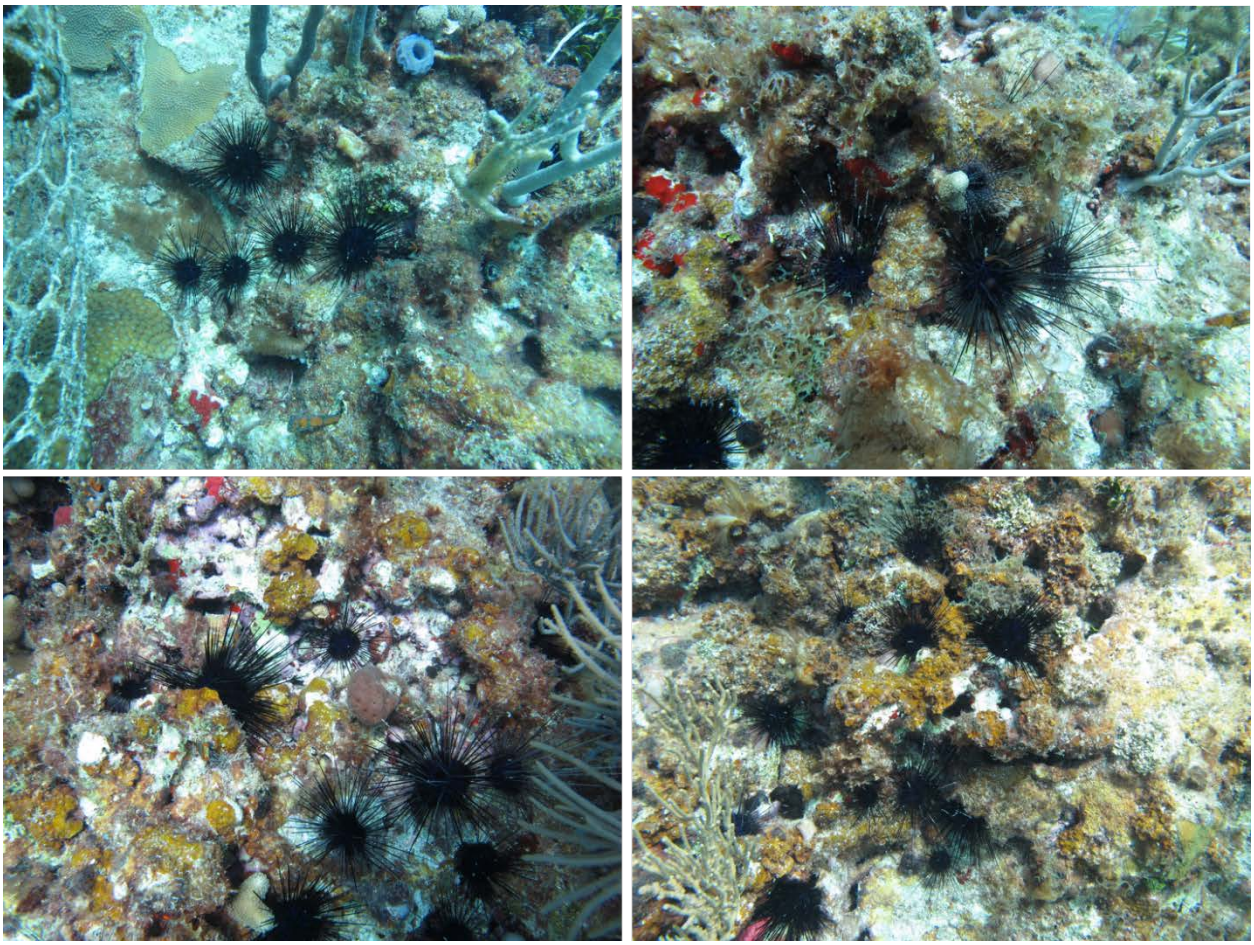


Figure 7 Pictures of the lab-reared Diadema antillarum restocked in the corral at Cayo Diablo and Los Lobos reefs in Fajardo, Puerto Rico.

Most of the *D. antillarum* escaped the corrals a day after the restocking. More sea urchins were retained in the corrals at Cayo Diablo than in Los Lobos. Seventeen urchins (11%) escaped at Cayo Diablo and 43 urchins (28.7%) escaped at Los Lobos (Table 2 and 3). Most of the escaped urchins were placed back into the fully enclosed corrals after one week. It was hard locating the sea urchins that we released because they were well hidden and may have moved from the area. However, we were able to locate some of the freed or escaped individuals one month after restocking because their grazing was visually evident on the surrounding reef surrounding. The

top was removed on one corral (Corral 1) at Los Lobos after one week, and most of the *D. antillarum* escaped in this corral.

Table 2 The number of restocked Diadema antillarum in closed and open corrals through time at Cayo Diablo.

Corrals with tops				Corrals without tops			
Date	**Corral 1**	**Corral 2**	**Corral 5**	**Date**	**Corral 3**	**Corral 4**	**Corral 6**
8/22/2018	25	25	25	8/22/2018	25	25	25
8/23/2018	25	25	25	8/23/2018	23	22	23
9/1/2018	20	15	16	9/1/2018	4	4	0
9/22/2018	16	12	11	9/22/2018	2	3	0
10/23/2018	9	0	8	10/23/2018	3	1	1

Table 3 The number of restocked Diadema antillarum in closed and open corrals through time at Los Lobos.

Corrals with tops				Corrals without tops				
Date	**Corral 1**	**Corral 4**	**Corral 5**	**Date**	**Corral 1**	**Corral 2**	**Corral 3**	**Corral 6**
8/22/2018	25	25	25	8/22/2018		25	25	25
8/23/2018	21	25	25	8/23/2018		12	19	22
9/1/2018		12	12	9/1/2018	5	1	4	0
9/22/2018		10	12	9/22/2018	2	1	2	0
10/23/2018		8	11	10/23/2018	0	1	2	0

As seen in Tables 2 and 3, the presence of *D. antillarum* through time varied between the type of corrals (closed or open). Overall, the presence in corrals was more significant in the closed corrals than in the open corrals. After the first month, an average of 44% and 52% or the restocked individuals were recorded in fully closed corrals in Los Lobos and Cayo Diablo, respectively. While after the first month, only 5% and 6% were recorded in open corrals at Los Lobos and Cayo Diablo. I did notice that the condition of the corrals after the first month significantly deteriorated. There were a lot of holes, and some of the plastic nettings was torn off. Small (damselfish and bluehead wrasse) to medium (surgeonfish) fish were observed many times inside the closed corrals. Two large spotted morays were counted in Corral 1 at Cayo Diablo and Corral 4 in Los Lobos by the second month of monitoring. It is unclear if the moray is a predator or *D. antillarum*.

There were only two dead sea urchins observed during this study, which occurred in a fully enclosed Corral 1 at Cayo Diablo.

The numbers of *D. antillarum* located in the corrals decreased even more so by the second month of monitoring. The majority of the corrals were destroyed and open (Fig. 8) by the second month. This was evident at Cayo Diablo and could be due to the wave or swell energy at this site since it is more exposed. No dead urchins, piles of spines, or skeletons were observed outside the corrals or the surrounding reef area during the entire monitoring. As witnessed by Williams et al. (2015), escaped individuals can move fast and far, about 20 to 30 meters from restocked areas. Therefore, it was theorized that the *D. antillarum* which could not be located inside the corrals were well hidden and/or far from the restocking area.

Figure 8 Photographs taken during the two-month monitoring showing the destructions of corrals at Cayo Diablo and Los Lobos reefs in Fajardo, Puerto Rico.

Benthic Cover

Pre-restocking

Before restocking, a visual assessment of *D. antillarum* populations was conducted at Cayo Diablo and Los Lobos. The density of *D. antillarum* around the corrals was low (< 1 Ind m^{-2}), with the exception at Los Lobos, where high densities of *D. antillarum* adults (~4 Ind m^{-2}) were observed about 50 m north of the corrals at shallower depths (< 2m).

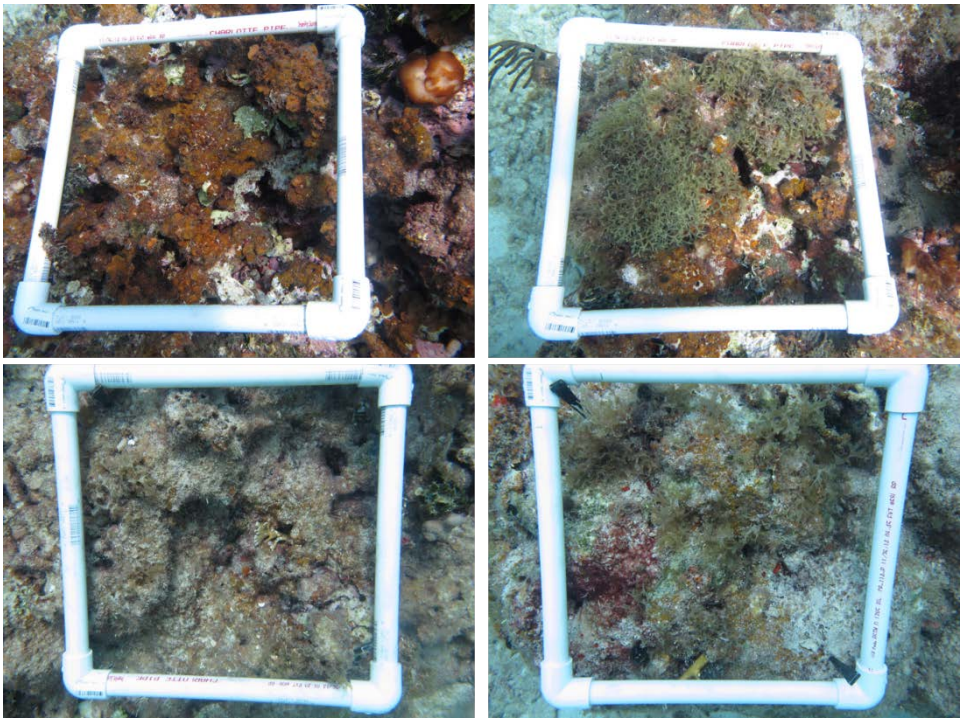


Figure 9 Photographs at the control station in Cayo Diablo (two photographs above random quadrat) and in Los Lobos (two photographs below fixed quadrats) in Fajardo, Puerto Rico.

The control station at Cayo Diablo, benthic algae was the dominant benthic substrate, with macroalgae (*Dictyota* spp.) and *Ramicrusta* contributing to the highest mean cover (mean ± se) in the fixed (56.7 ± 9.4%) and random quadrats (63.3 ± 9.3%), respectively. At Los Lobos,

Ramicrusta was the main contributor to the overall algal composition, with mean cover ranging from 39.7 ± 10.2% in random quadrats and 31.3 ± 3.2% in permanent quadrats.

In the corrals, the benthic composition was similar to that in the control station and was mainly characterized by the high cover of *Ramicrusta* at both Cayo Diablo and Los Lobos, ranging from 41.8 ± 11.3% to 54.2 ±11.0% and from 62.8 ± 9.3% to 83 ± 5.6%, respectively (Fig. 9). Turf algae were also prominent outside and inside corrals. Turf was both sparse and filamentous and or in thick mats (algal mats), which allowed for the capture of sand and other sediments. Inside the corrals, the cover of other benthic organisms was low, especially that of coral cover, which ranged in cover between 0.11 ± 0.1% to 13.9 ± 8%at Cayo Diablo to 0% to 12.75 ± 6.7% at Los Lobos.

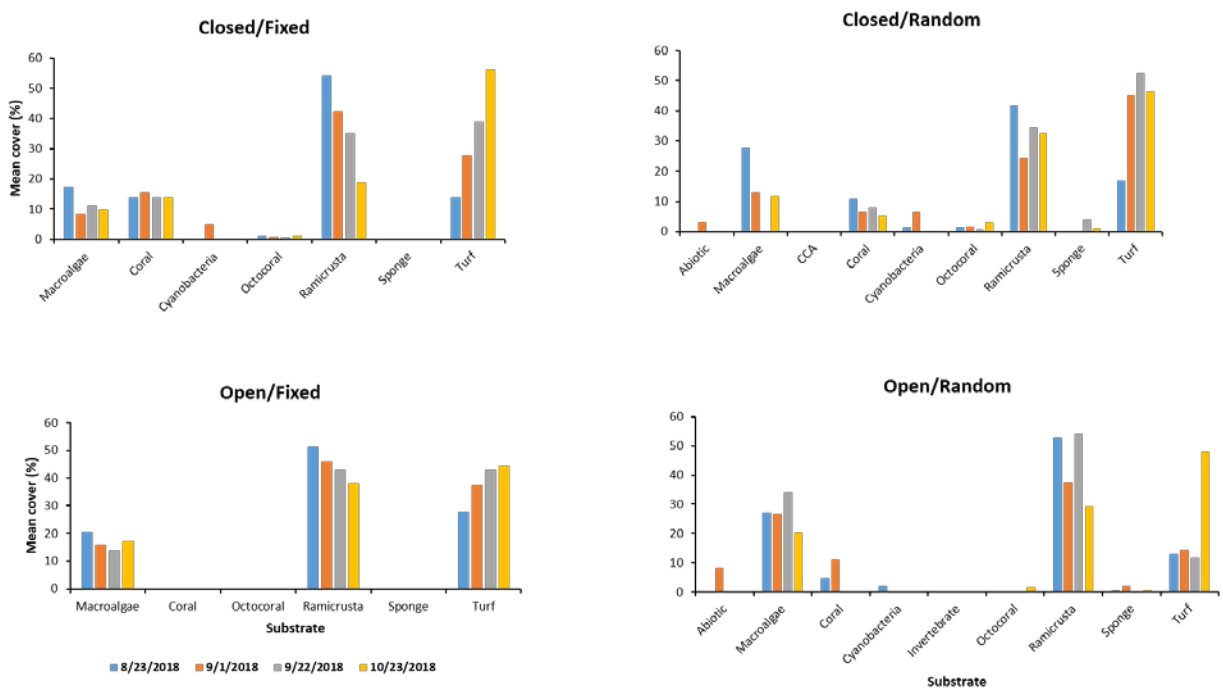


Figure 10 The mean cover of benthic organisms in the corral type (open and closed) and quadrat type (fixed and random) through the sampling events at Cayo Diablo, Fajardo.

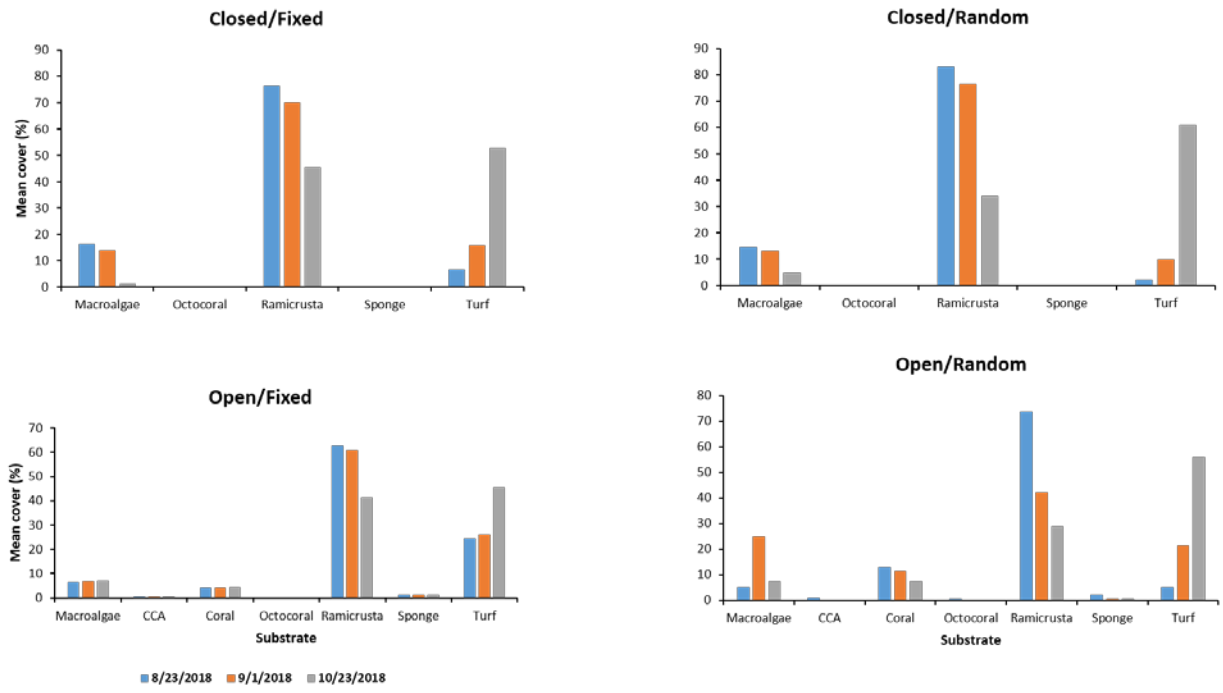


Figure 11 The mean cover of benthic organisms in the corral type (open and closed) and quadrat type (fixed and random) through the sampling events at Los Lobos, Fajardo.

Restocking effects

The benthic composition did not vary at the control stations through the monitoring period (Table 4). Algal cover, specifically *Ramicrusta* and turf algae, continued to be the dominant substrate outside the corrals through the monitoring period. There was no grazing observed in the control area. Therefore, it can be assumed that the changes observed in the benthic cover inside the corrals, both open and closed, are due to the grazing of restocked *D. antillarum*.

Table 4 The results of a three-way distance Permutational Multivariate Analyses of Variance (PERMANOVA) to detect changes in benthic composition at the control stations between sites (Si), quadrat types (Qu: fixed or random), and sampling dates (Da).

Source	df	SS	MS	Pseudo-F	p value
Si	1	6414.4	6414.4	4.8184	0.015
Qu	1	2834.7	2834.7	2.1294	0.106
Da	3	3336.1	1112	0.83533	0.59
SixDa	2	1865.5	932.76	0.70068	0.618
SixQu	1	1614.9	1614.9	1.2131	0.331
QuxDa	3	3336.1	1112	0.83533	0.601
SixQuxDa	2	1865.5	932.76	0.70068	0.596

One week after the restocking, the benthic substrate was characterized by more turf algae, and less *Ramicrusta* (see Fig. 12). *Ramicrusta* was reduced by as much as 57% at Cayo Diablo and 43% at Los Lobos. Also, there was also a lower abundance of fleshy macroalgae, specifically *Dictyota* spp, after one week. Even though the abundance of *Ramicrusta* was reduced after one week (see Fig. 13), the reduction was not significant for all corrals. Therefore, resulting in the insignificant statistical results of the overall grazing effects of *D. antillarum* on the benthic composition. These results are similar to the restocking efforts in 2016 at Media Luna in La Parguera, Puerto Rico (Williams 2017). Grazing was evident after one week, however only ~20% was effectively grazed of algae after one week. Differences in the benthic composition might have been more significant if there was higher retention of restocked individuals in the corrals.

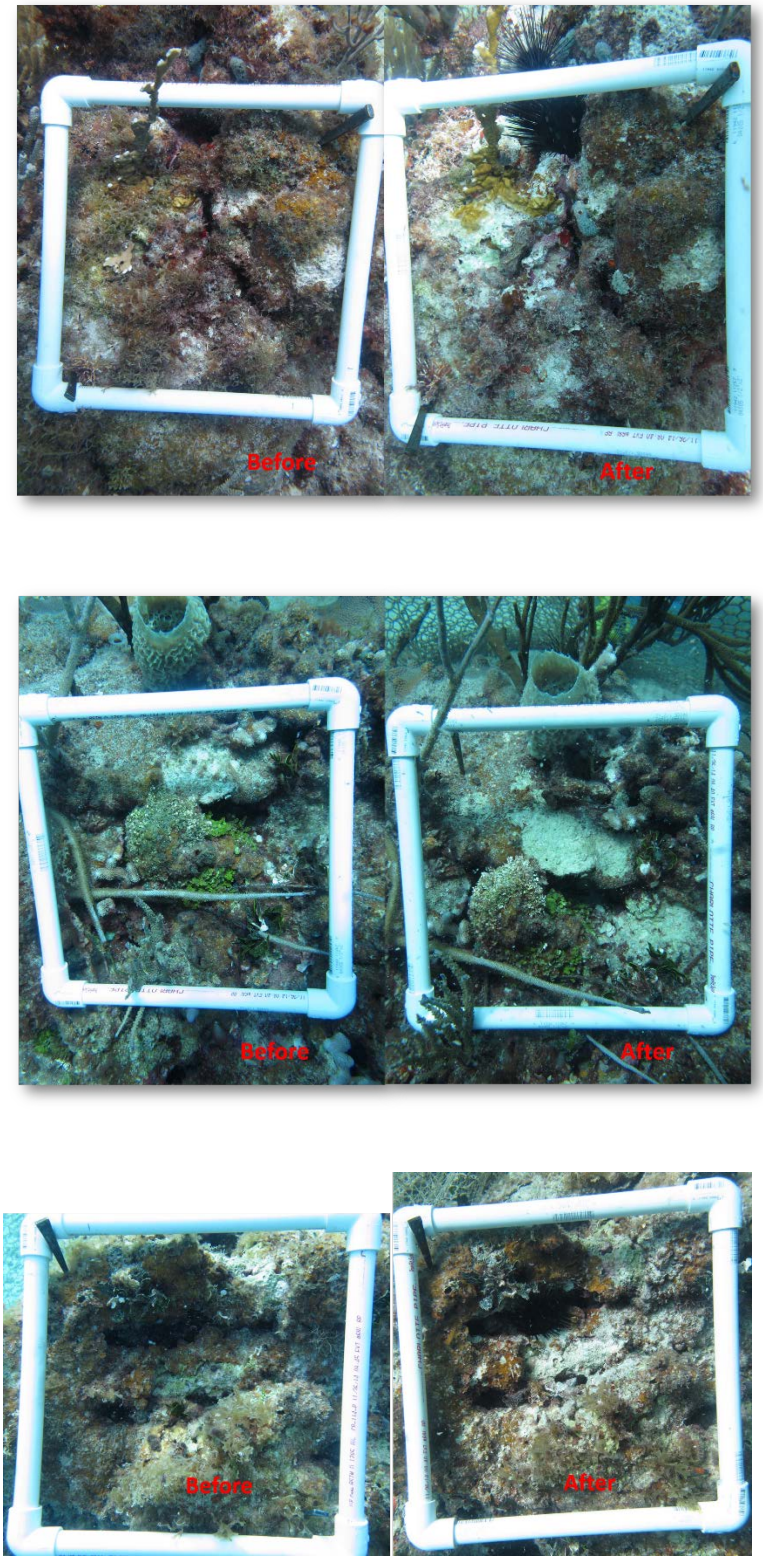


Figure 12 Photographs of the fixed quadrats before and one week after the restocking of Diadema antillarum at Cayo Diablo (first two sets of photographs) and Los Lobos (third set of photograph) in Fajardo, Puerto Rico.

Benthic change was more evident in the closed corrals, given there was more grazing pressure. The abundance of *D. antillarum* recorded in an open corral was low and the retention ranged from one to three individuals (< 1 Ind m^{-2}). The grazing pressure was still evident in the open corrals, however it was limited to the area of homing, thus explaining the lack of significance in the PERMANOVAs. Densities of one individual per square meter is not uncommon on coral reefs around the Caribbean. The majority of reefs are characterized by even lower densities. As seen in this study, the densities of individuals, < 1 Ind m^{-2}, did not significantly impact the benthic composition after two months. However, this might have changed with a more extended monitoring period. Future *D. antillarum* restoration projects should focus on restoring populations greater than one individual per square meter.

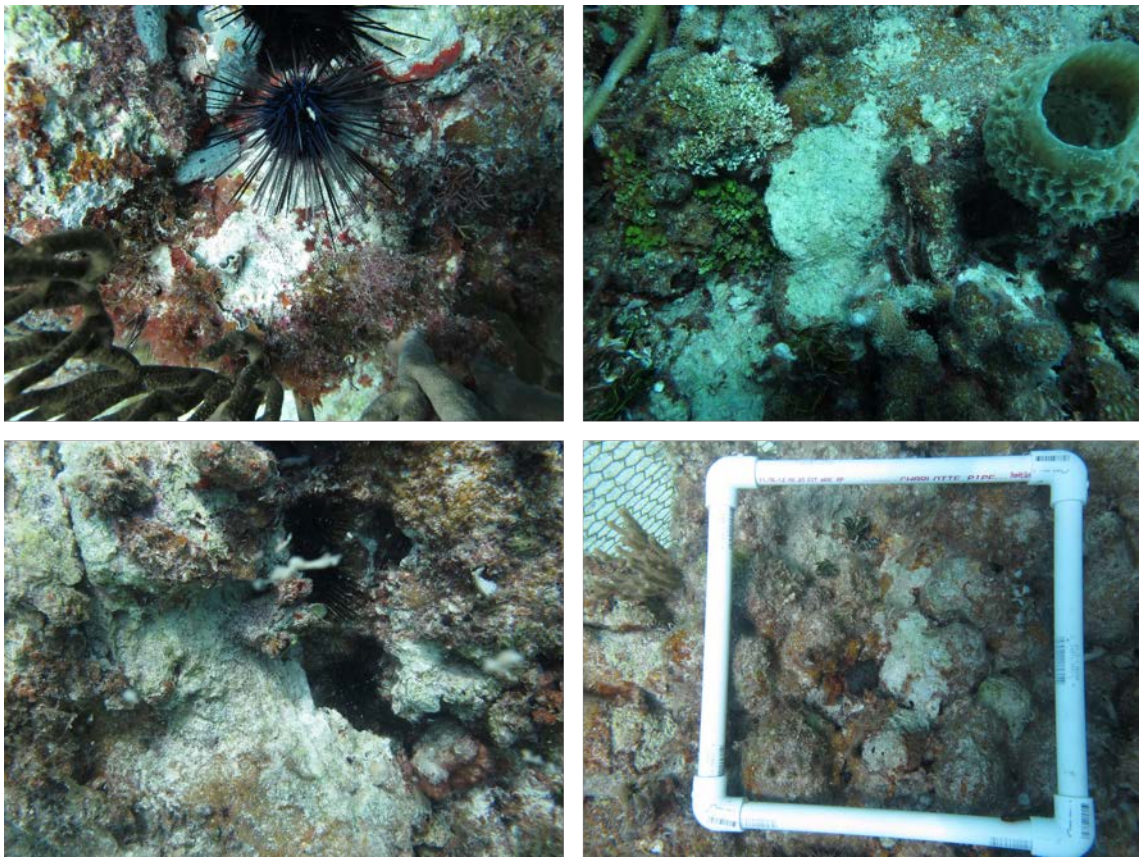


Figure 13 Photographs taken to highlight the grazing of restored Diadema antillarum one week after the restocking at Cayo Diablo and Los Lobos in Fajardo, Puerto Rico.

As seen in the PERMANOVA tables (Indices 1-2) and the Principal Coordinate Analysis (PCA, Figure 14 and 15), there was a shift in benthic community structure after one and two months after the restocking (9/22/2018, 10/23/2018). The difference in benthic assemblage after one month was due to the reduction of *Ramicrusta* cover. The abundance of *Ramicrusta* was significantly reduced over the monitoring period (Indices 3-4). This pattern was valid in both the fixed and random quadrats at each site. In the closed corrals and during the two-month monitoring, *Ramicrusta* cover ranged from 18.9% to 32.7% at Cayo Diablo and from 33.8% to 45.7% at Los Lobos. The reduction in *Ramicrusta* was slightly more evident at Cayo Diablo than at Los Lobos; where *Ramicrusta* was reduced as much as 63% at Cayo Diablo (Fig. 16) and 61% at Los Lobos (See Indices 5 and 6). After one and two months, the substrate inside the corrals was characterized by more available space, turf algae, for corals and other benthic organisms to settle and colonize. Coral settlers were not observed during the monitoring period, which is not surprising since monitoring lasted for two months. The evidence presented in this study supports that the action of restocking of *Diadema antillarum* may be useful mitigation tool to control *Ramicrusta* and other algal abundance on the coral reefs.

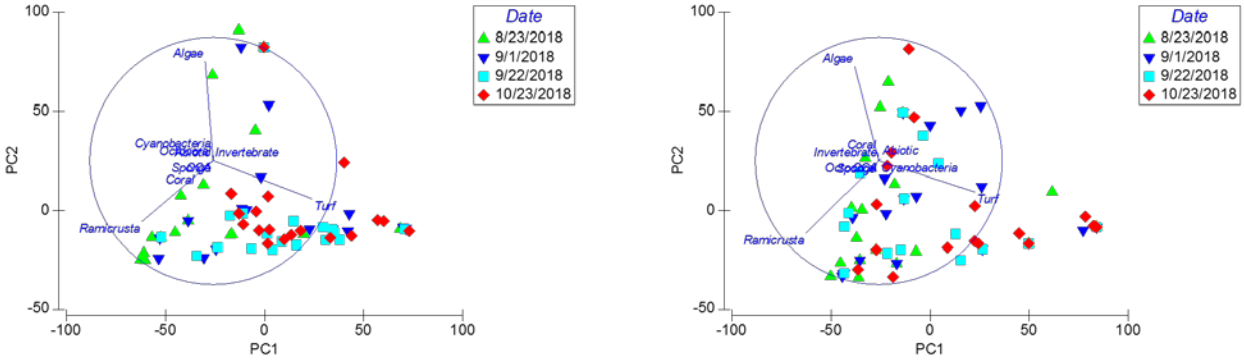


Figure 14 Principal Coordinate Analysis on the benthic composition of closed (left) and open (right) corrals at Cayo Diablo during the sampling periods. Algae represents all macroalgae.

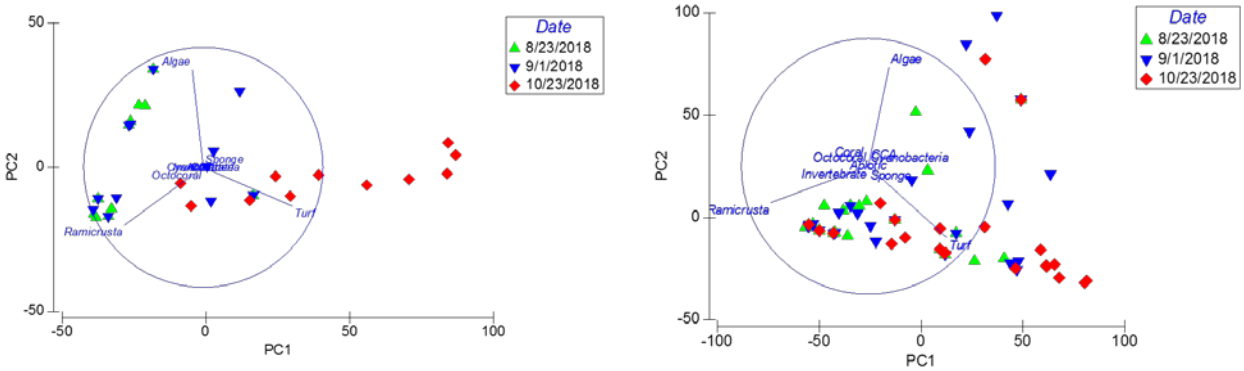


Figure 15 Principal Coordinate Analysis on the benthic composition of closed (left) and open (right) corrals at Los Lobos during the sampling periods. Algae represents all macroalgae.

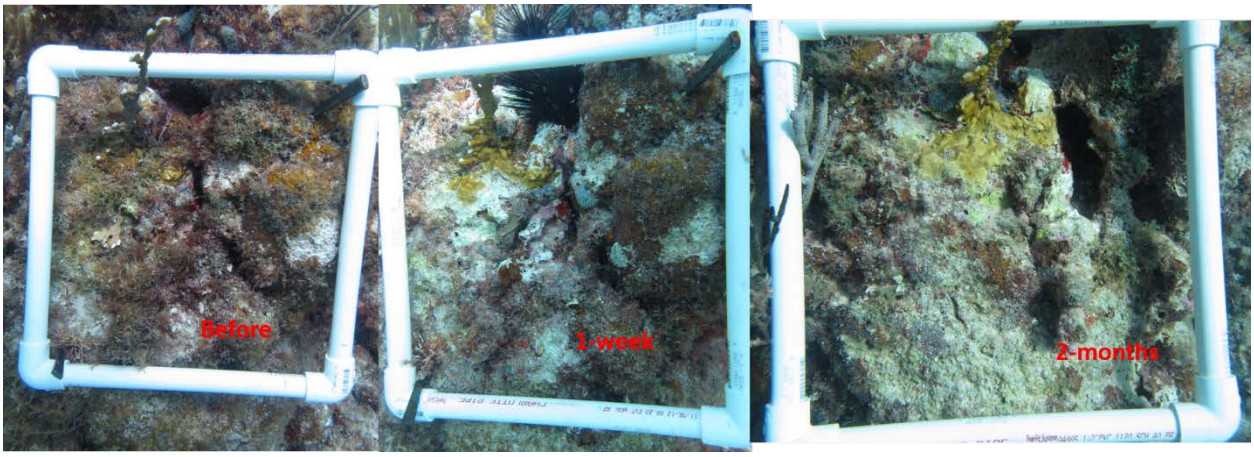


Figure 16 Photographs of a fixed quadrat in Corral 5 at Cayo Diablo before, one week and two months after the restocking of Diadema antillarum.

Conclusions and recommendations

- Only a few dead sea urchins were witnessed during the monitoring period. Therefore, it was assumed that the sea urchins escaped the corrals. Some of the escaped sea urchins were located one month after the restocking. Therefore, the survivorship of the restocked sea urchins might be high.
- Fully enclosing corrals are more effective in retaining the sea urchins for the monitoring period. The sea urchins escape open corrals and this usually occurs during the first couple weeks.
- It is necessary to fully enclose the corrals in order to effectively assess the change in benthic composition.
- As witnessed by Dr. Williams in laboratory and in this study, *Diadema antillarum* consume *Ramicrusta* spp. and other algae that can be a nuisance on a coral reef, *Dictyota* spp.
- *D. antillarum* are effective in reducing the *Ramicrusta* on a coral reef.
- Grazing impacts on algal cover are significantly evident after one month of restocking. However, this time scale might vary depending on the amounts of sea urchins in a given space.
- Restocked densities must be greater than 1 Ind m^{-2} in order to observe significant benthic change on a coral reef.
- Given the results of this study, the restocking of *D. antillarum* might be an effective mitigation tool in the reductions of algal cover, especially *Ramicrusta*.

Indices

Index 1 The results of a three-way distance Permutational Multivariate Analyses of Variance (PERMANOVA) to detect changes in benthic composition between corral type (Co: open or closed), quadrat types (Qu: fixed or random), and sampling dates (Da) at Cayo Diablo, Fajardo.

		Cayo Diablo			
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p value**
Co	1	8597.5	8597.5	7.6289	0.002
Qu	1	1830.5	1830.5	1.6243	0.193
Da	3	13688	4562.7	4.0487	0.002
CoxQu	1	3468.1	3468.1	3.0774	0.038
CoxDa	3	2708.4	902.79	0.80109	0.577
QuxDa	3	1977	659.01	0.58477	0.748
CoxQuxDa	3	5079.8	1693.3	1.5025	0.164

Index 2 The results of a three-way distance Permutational Multivariate Analyses of Variance (PERMANOVA) to detect changes in benthic composition between corral type (Co: open or closed), quadrat types (Qu: fixed or random), and sampling dates (Da) at Los Lobos, Fajardo.

		Los Lobos			
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p value**
Co	1	4750.6	4750.6	5.2569	0.01
Qu	1	132.27	132.27	0.14636	0.865
Da	2	23099	11550	12.78	0.001
CoxQu	1	362.62	362.62	0.40127	0.689
CoxDa	2	187.53	93.763	0.10376	0.949
QuxDa	2	1662.8	831.39	0.91999	0.454
CoxQuxDa	2	1666.4	833.21	0.92201	0.463

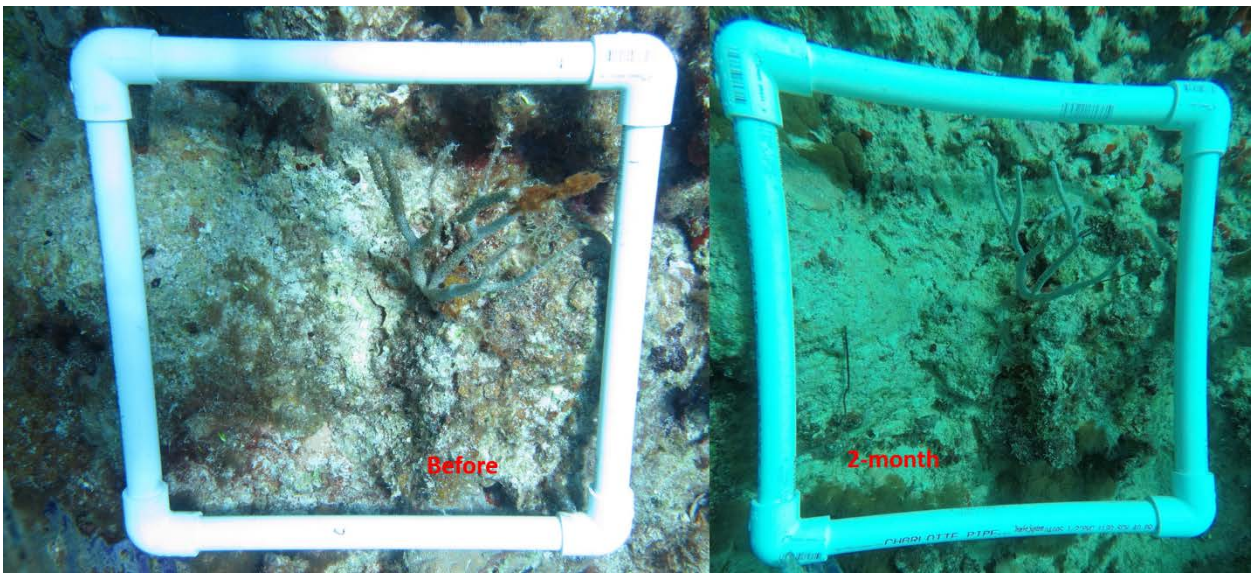
Index 3 The results of a three-way distance Permutational Multivariate Analyses of Variance (PERMANOVA) to detect changes in Ramicrusta spp. between corral type (Co: open or closed), quadrat types (Qu: fixed or random), and sampling dates (Da) at Cayo Diablo, Fajardo.

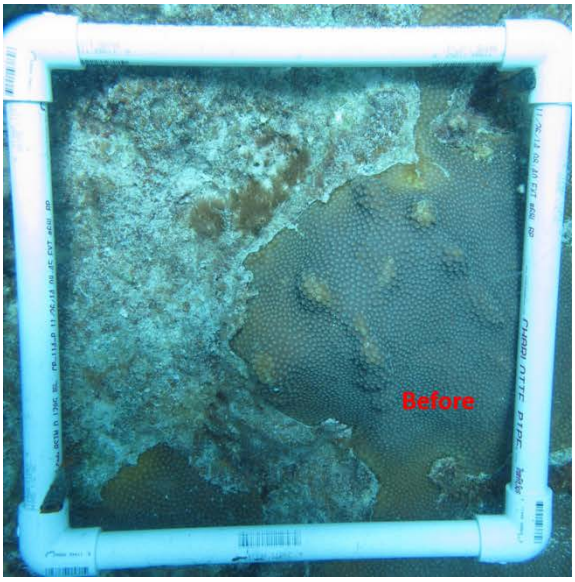
		Cayo Diablo			
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p value**
Co	1	2492.7	2492.7	3.2456	0.077
Qu	1	278.77	278.77	0.36298	0.542
Da	3	7577.8	2525.9	3.2889	0.026
CoxQu	1	87.728	87.728	0.11423	0.727
CoxDa	3	378.61	126.2	0.16433	0.908
QuxDa	3	1838.6	612.87	0.79799	0.483
CoxQuxDa	3	1982.3	660.77	0.86037	0.446

Index 4 The results of a three-way distance Permutational Multivariate Analyses of Variance (PERMANOVA) to detect changes in Ramicrusta spp. between corral type (Co: open or closed), quadrat types (Qu: fixed or random), and sampling dates (Da) at Cayo Diablo, Fajardo.

		Los Lobos			
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p value**
Co	1	3842.2	3842.2	4.6252	0.042
Qu	1	255.67	255.67	0.30777	0.567
Da	2	22300	11150	13.422	0.001
CoxQu	1	330.04	330.04	0.3973	0.511
CoxDa	2	1180	590.02	0.71026	0.479
QuxDa	2	1848.3	924.13	1.1125	0.315
CoxQuxDa	2	1016.8	508.39	0.61199	0.523

Index 5 Photographs of permanent quadrats before and two months after the restocking of Diadema antillarum in Cayo Diablo and Los Lobos, Fajardo, Puerto Rico.

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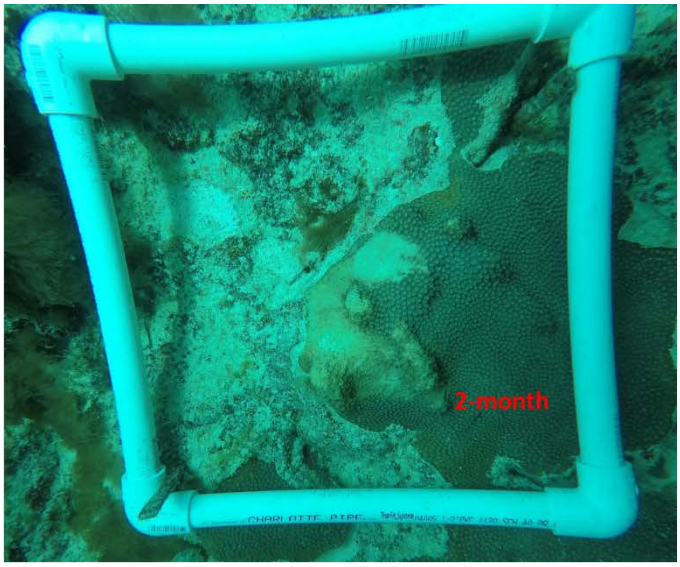
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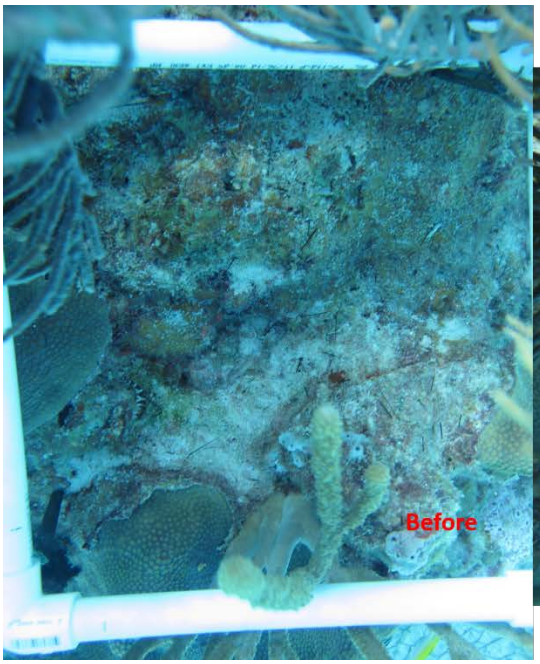
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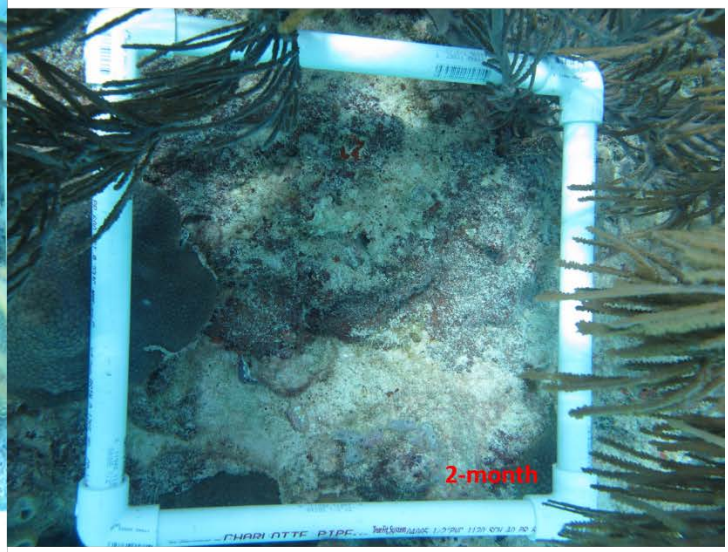
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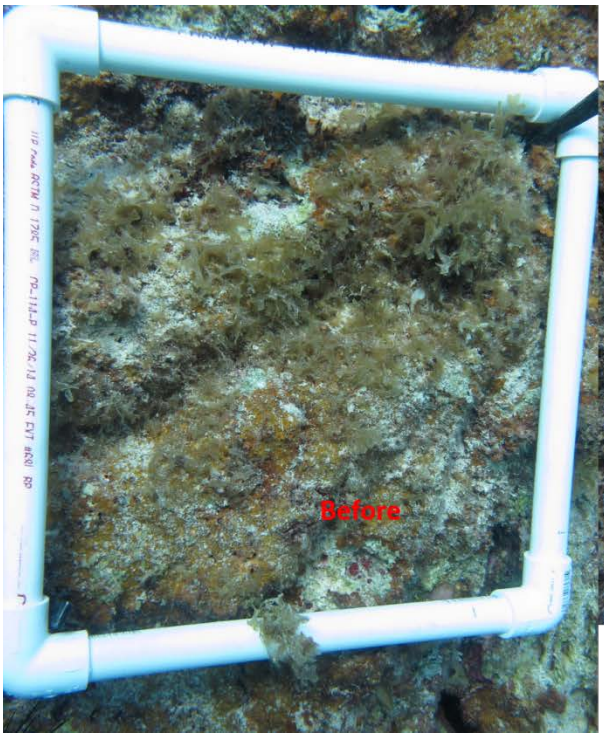
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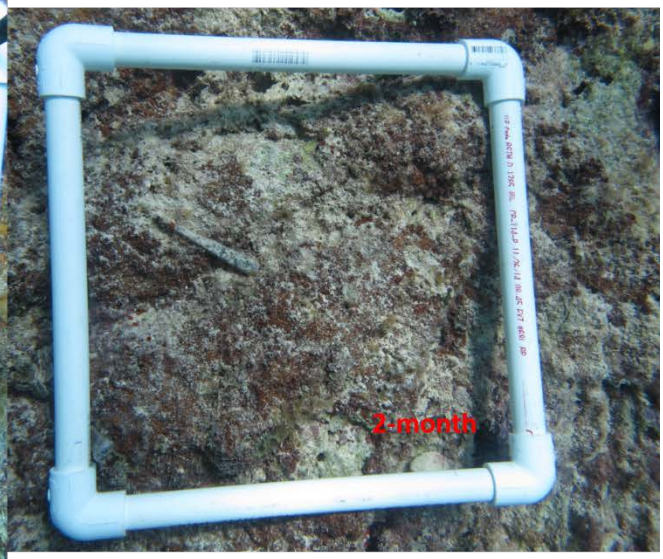
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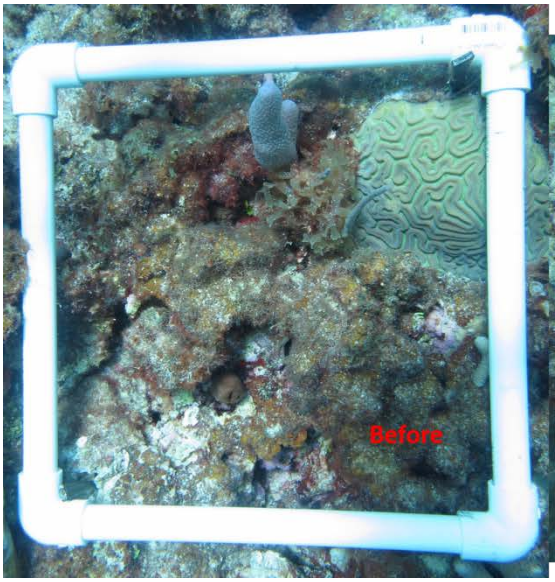
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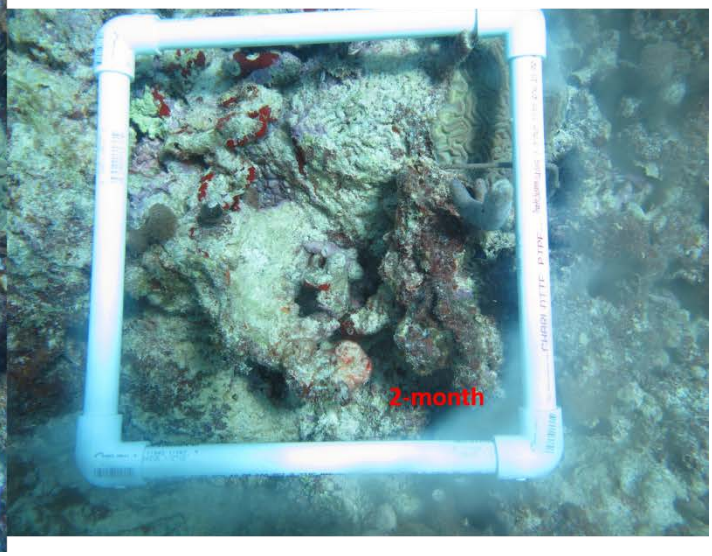
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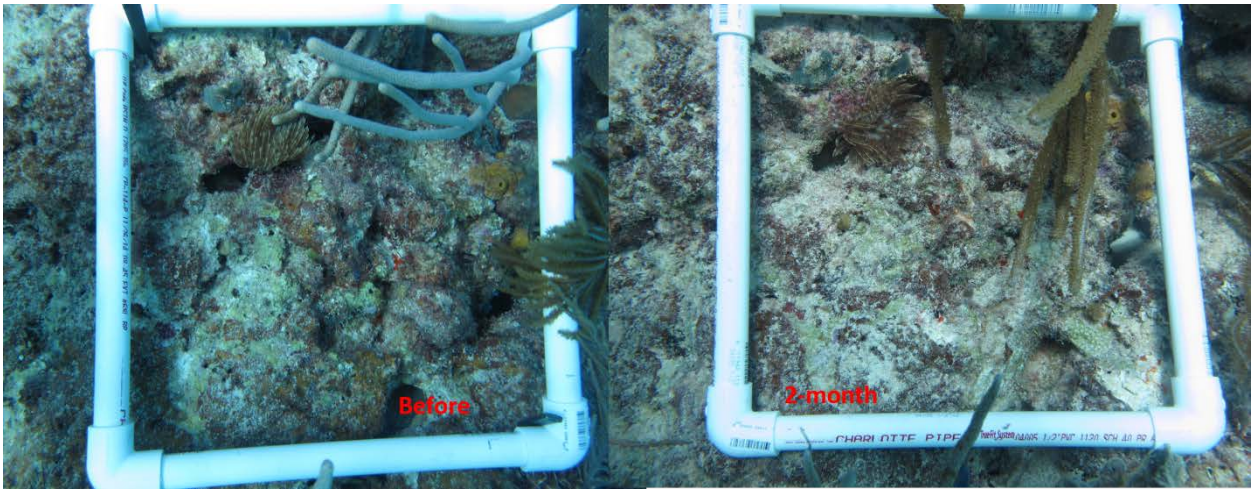
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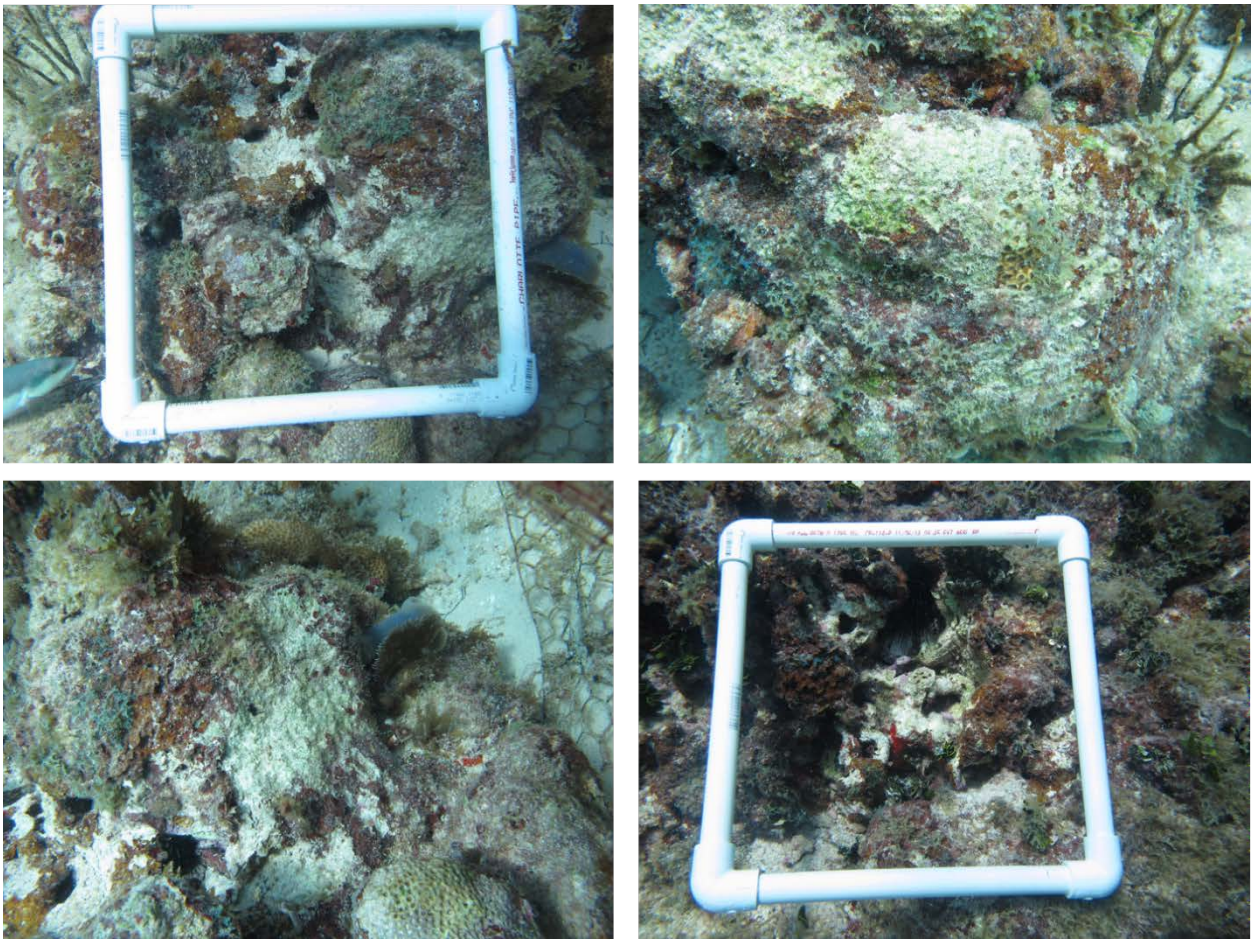
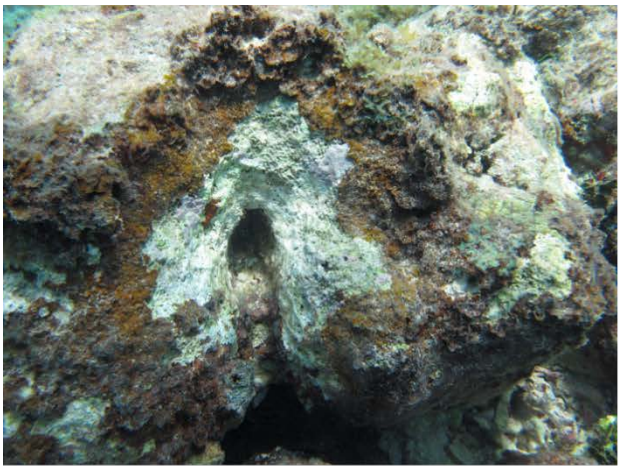
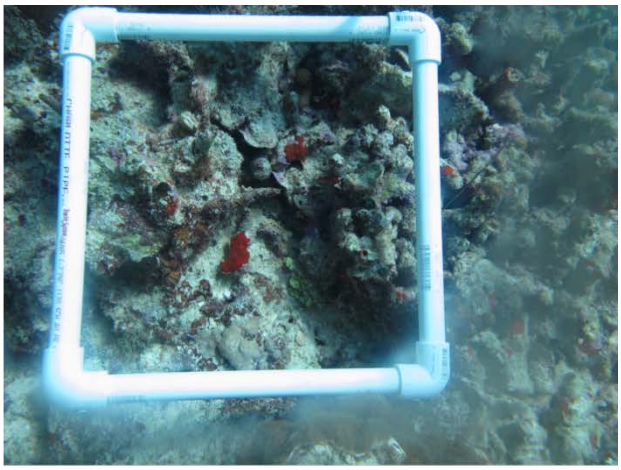
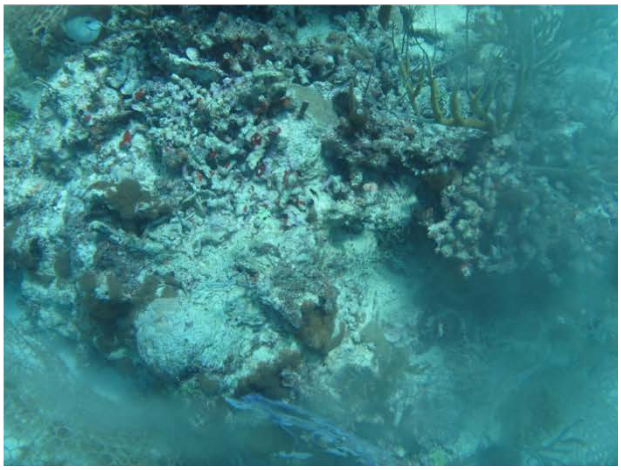
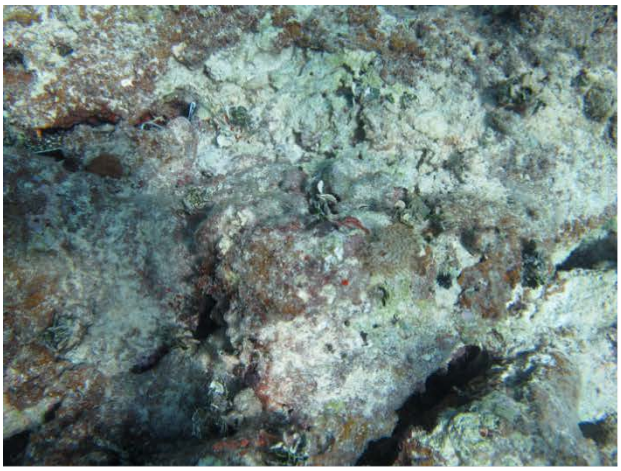
Index 6 Photographs of the grazing effects of Diadema antillarum two months after restocking in the corrals at Cayo Diablo and Los Lobos, Fajardo.

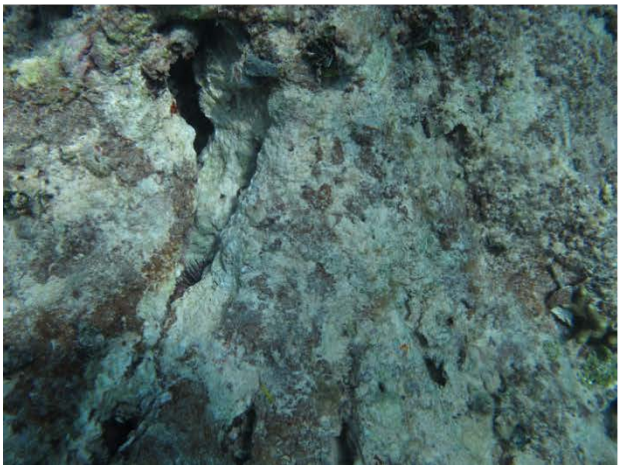


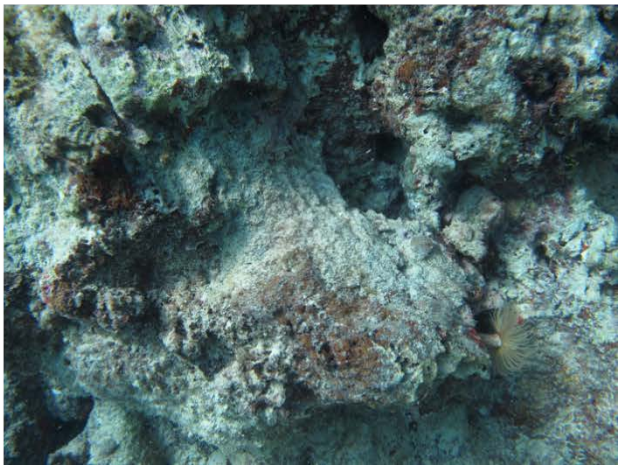










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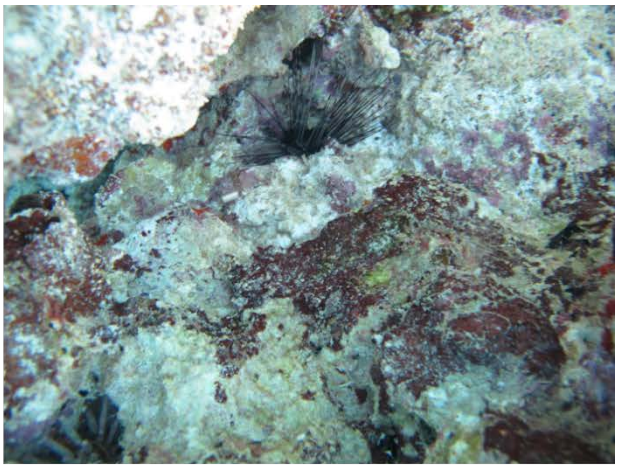

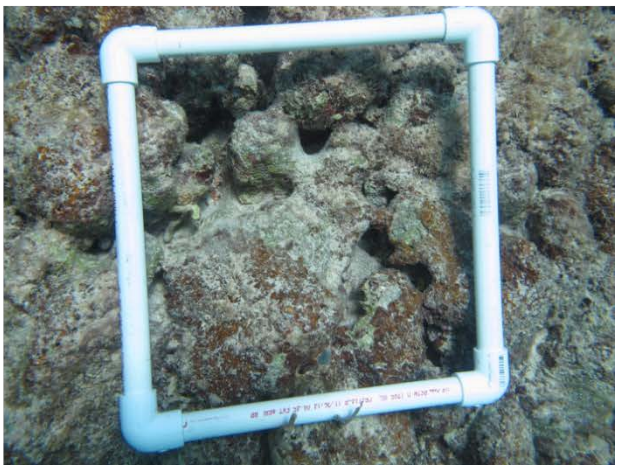

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