The control of algal abundance on coral reefs through the reintroduction of *Diadema antillarum*

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Executive Summary

The dominant benthic substrate on many coral reefs in Puerto Rico consists of fleshy macroalgae and turf algae, especially turf with sediment. Fleshy macroalgae and turf with sediment can be detrimental for the settlement of post-larval corals and growth and survivorship of juvenile corals. Fleshy macroalgae, especially *Dictyota* spp. can retard growth rates of juvenile corals by outshading and abrasion (Box and Mumby 2007). The allochemicals produced by brown, fleshy macroalgae, can also introduce dangerous bacteria and inhibit coral larvae to settle (Morrow et al. 2017). Turf, which is short, filamentous and without sediment, will not affect the settlement and survivorship of corals (O'Brien and Scheibling 2018). However, quite often algal turf will accumulate sediments when there is a terrestrial source close by or there is constant resuspension of sediments. Birrel et al. (2005) found that coral settlement was significantly reduced when algal turf accumulated sediments. The objective of this project was to increase herbivory rates on two coral reefs in La Parguera and decrease the algal abundance, specifically fleshy macroalgae and turf with sediment.

A total of 6,205 *D. antillarum* settlers were collected during the summer of 2018. The settlement in 2018 was the highest compared to the collections of past years. Settlers were brought back to the laboratory at the University of Puerto Rico on Magueyes Island, 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 limited space in the tanks and the change in water quality measurements, such as salinity and temperature.

Restocking efforts were modified due to the passing of tropical storm, Dorian, which was on the path to impact the southwest of Puerto Rico in August 2019. The storm was forecasted to bring at least 50 mph winds and might reach a category one hurricane before making landfall. The

tarps at the grow-out facility were removed. There were other complications with the passing of the storm, such as the possible loss of electricity and the lack of access to the island. The risks were weighed and an a emergency amendment was requested to the Department of Natural and Environmental Resources to transplant the sea urchins to a sheltered coral reef in La Parguera. On August 27, 2019 the lab-reared urchins were transferred to the back reef of Media Luna, La Parguera. A total of 480 sea urchins were placed on the reef without any corrals. Some of these sea urchins were then retrieve and transferred to El Coral and Mario on August 31, 2018.

On August 31, 2019, 276 lab-reared sea urchins were retrieved from Media Luna and were transferred to two reefs in La Parguera, El Coral and Mario. Twenty-three urchins were placed in six corrals that were installed at each reef and fully enclosed (with tops). Many of the tops were broken by octocorals and/or possibly fish after the first week of monitoring. Therefore, most of the *D. antillarum* escaped the corrals a week after the restocking. All tops were removed during the one month monitoring. After the first month, retention of individuals ranges from 26% to 78% at El Coral and 0% to 26% at Mario. The numbers of *D. antillarum* located in the corrals decreased even more so by the second month of monitoring.

The dominant benthic substrate at the control stations at both La Parguera stations during the monitoring was fleshy (*Dictyota* spp., *Padina* spp., etc.) and articulate coralline algae (*Halimeda* spp., *Jania* spp., etc.). The benthic cover significantly changed over time at the control station at El Coral because the abundance of *Dictyota* spp. significantly increased by the second month by 460%. Even though the benthic cover did not significantly changed over time at Mario, there was also a significant increase in *Dictyota* spp. of 272%. The other common substrates at both control sites were turf and turf with sediment.

At both El Coral and Mario, the grazing effects of *D. antillarum* significantly changed the benthic composition inside the corrals. Even after one week of the restocking, the grazing effects of *D. antillarum* were evident at El Coral and Mario. *Dictyota* spp. cover was reduced between 52% and 54% at El Coral, and between 46% and 52% at Mario during the first week of restocking the sea urchins. By the second month after restocking, *Dictyota* spp. cover was reduced up to 90%, and turf with sediment, another unsuitable substrate, was reduced between 77% and 92% at El Coral. Even though most sea urchins escaped the corrals at Mario, the impacts of restocking *D. antillarum* were still significant by the two month monitoring. At both sites, the reef substrate was characterized by more suitable substrates for coral settlement, such as small, filamentous turf, crustose coralline algae (CCA), and clean substrate ("pavement") after two months of restocking. For example, small, filamentous turf algae, and CCA and clean substrate ("pavement") increased through the sampling period by 93.3%, 98.2%, and 99.2%, respectively at El Coral. The evidence presented in this study supports that the action of restocking of *Diadema antillarum* confirms that this sea urchin is a useful mitigation tool to control algal abundance on the coral reefs.

Contents

Figure List

Table List

Index List

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, Tuohy et al. 2020), nevertheless, densities are still far below pre-mass mortality numbers (Lessios 2016). In La Parguera, Tuohy et al. (2020)

observed no significant increase in *D. antillarum* populations from 2001. In addition, populations were dominated by medium to large (5-9 cm test diameter) individuals and were concentrated at shallower (< 5m), more complex reefs. 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). Algae not only is a threat to slow growing sessile-benthic organisms but has the potential to reduce the area of suitable substratum for coral settlement.

The goal of this project was to increase herbivory rates at two coral reefs in La Parguera, El Coral and Mario, to decrease unsuitable substrates, such as fleshy macroalgae and turf with sediments.

Methods

Collection

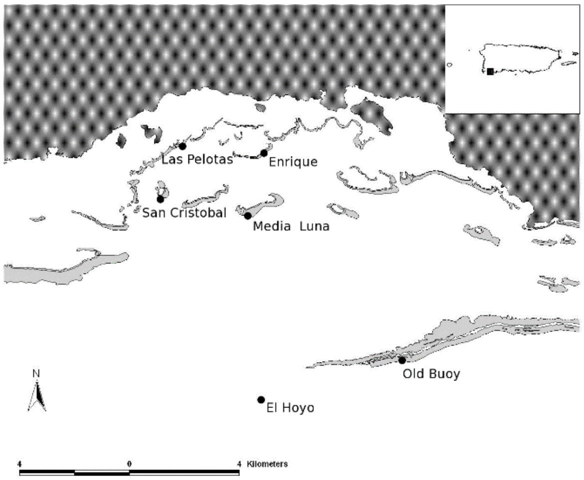

Figure 1. Settlement sites in La Parguera, Puerto Rico

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. 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}). Twenty mooring lines were placed at Old Buoy and ten mooring lines were placed at El Hoyo. On each mooring line, a rope containing 20, 10 × 10 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 clean plates was attached.

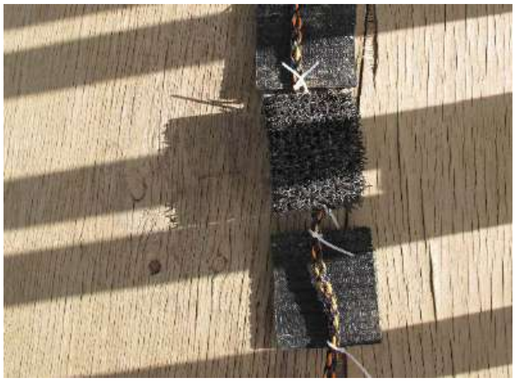

Figure 2. Astroturf or artificial turf used as settlement

Settlement plates were replaced each month from June to October 2018 (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 (Fig. 4). All tanks were on a fully closed system, to improve survivorship. 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 through a filter sock (200 micron) and run through a UV light. Water quality measurements, like salinity, temperature, and pH were recorded weekly. Other measurements like nitrate and ammonium were measured on a monthly basis or when needed. Settlers were transferred to wet tables or raceways (Fig. 5) once they reached a size of 5-6 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 (200 microns). The

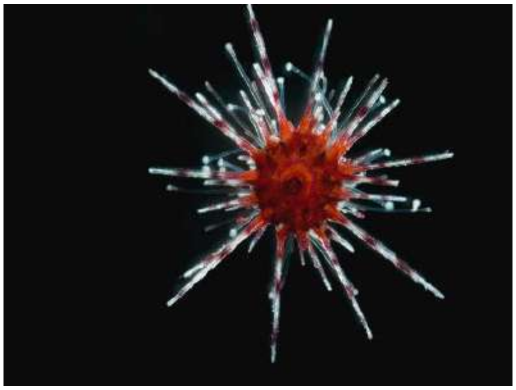

Figure 4. Diadema antillarum settler

Figure 3. The closed tank system for the small Diadema antillarum settlers which is located at the University of Puerto Rico, Mayagüez.

sock was cleaned and replaced once a week and raceways were cleaned once every two weeks.

Figure 5. Wet laboratory at the University of Puerto Rico, Mayagüez.

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

Restocking

The initial plan for the lab-reared *Diadema antillarum*, was to transplant them to two reefs in Culebra Island. However, the plans had to be modified because there was a tropical storm, Dorian, on the path to impact the southwest of Puerto Rico by Wednesday, August 28, 2019. The storm was forecasted to bring at least 50 mph winds and might reach a category one hurricane before making landfall. The tarps at the grow-out facility had to be removed, which would have left the sea urchins vulnerable to low salinity conditions because the storm was supposed to bring a lot of rain. There were other complications with the passing of the storm, such as the loss of electricity (no flow in the tanks) and the lack of access to the island. The NOAA Restoration Center was consulted and the risks were weighed. An emergency amendment was requested to the Department of Natural and Environmental Resources to transplant the sea urchins to a safe location in La Parguera. On August 27, 2019 the lab-reared urchins were transferred to the back reef of Media Luna, La Parguera. A total of 480 sea urchins were placed on the reef without any corrals

(Fig. 6). These sea urchins were then retrieve and transferred to El Coral and Mario on August 31, 2018 (see below).

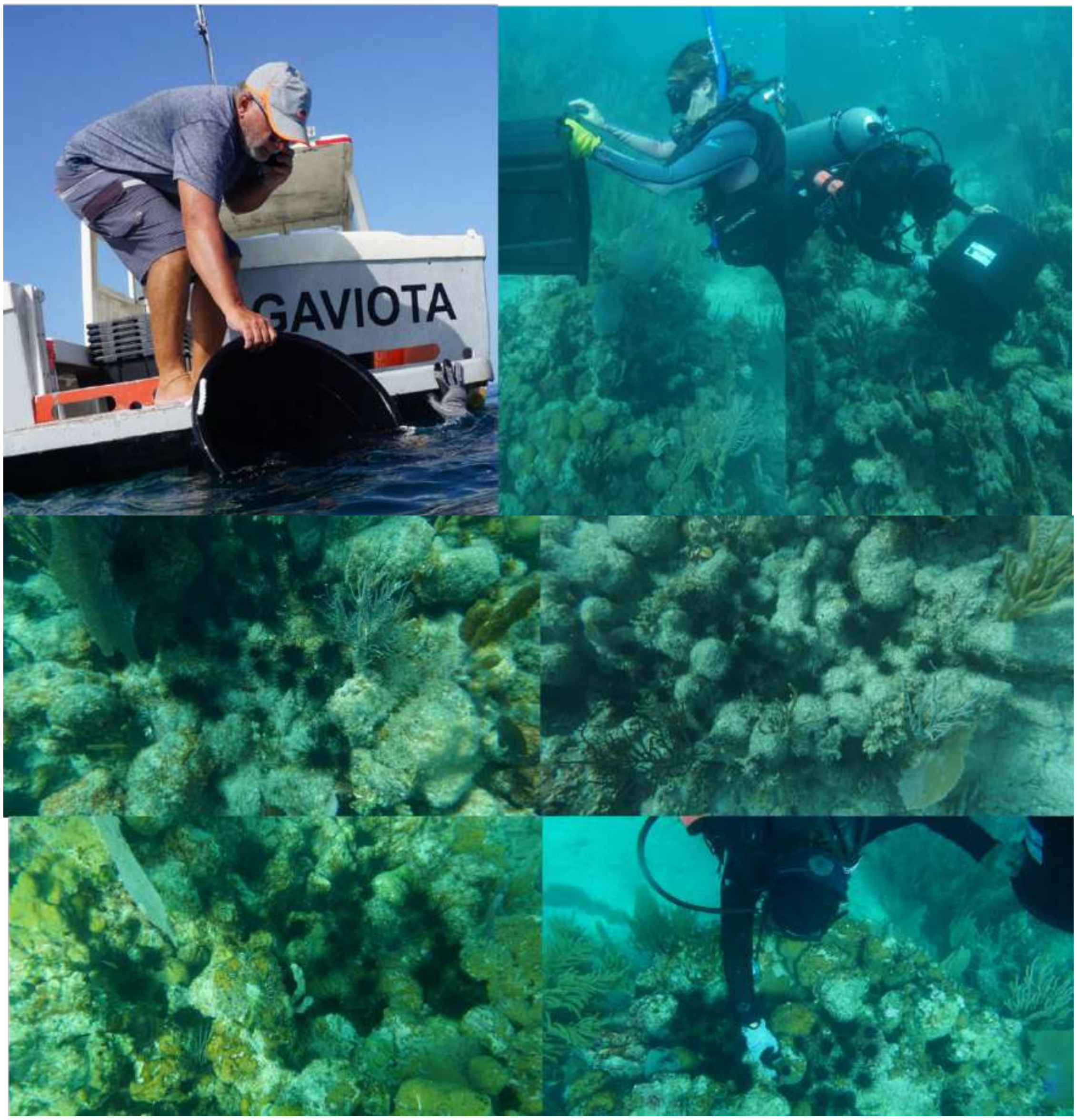

Figure 6. Photographs taken of transporting and transplanted lab-reared Diadema antillarum to a back reef, Media Luna, in La Parguera.

On August 30, 2019 six corrals were installed at El Coral (17.94864, -67.01798) and another six were installed at Mario (17.95312, -67.05634), La Parguera (Fig. 7). Corrals (Fig. 8-9) were installed at each reef in 3 meters of depth at El Coral and at 3-6 meters at Mario. Corrals were 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. Tops were installed on all of the corrals to enclose the area fully and limit the escape of sea urchins, as seen in Fajardo in 2018.

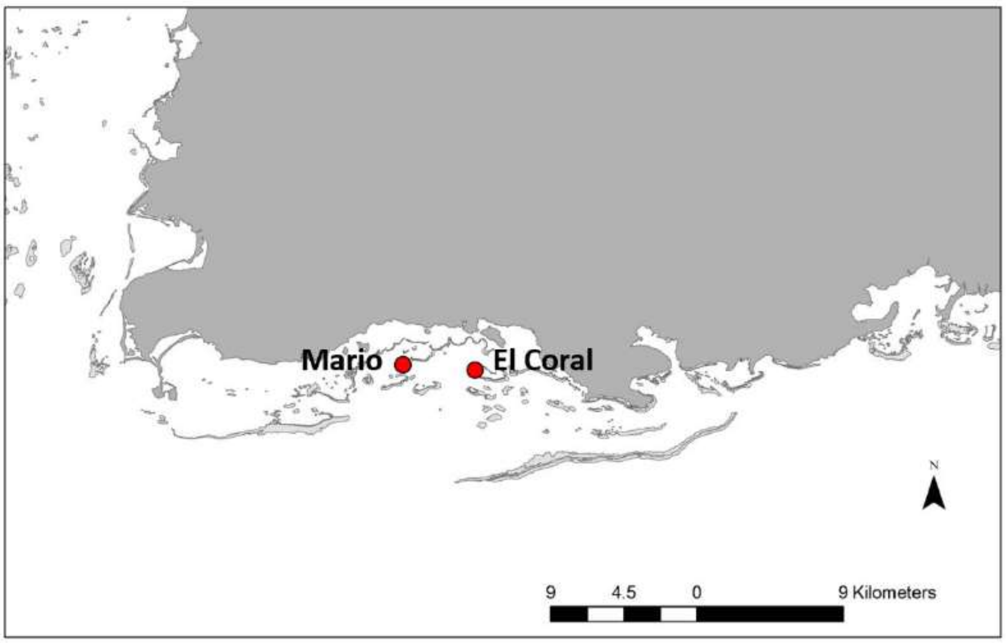

Figure 7. Map of the restoration sites, El Coral and Mario in La Parguera, Puerto Rico.

Initial benthic assessments of each corral took place on August 30, 2019. 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. 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. Fixed quadrats were placed in areas with high algal cover. Nails were 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. The control station was monitored for the last two months of the monitoring. Percentage cover of algae was discriminated to the lowest possible taxonomic level. The photographs were examined in the laboratory and the relative percentage cover of sessile organisms was estimated using Coral Point Count with Excel extensions. In CPCe, 50 points were placed in a uniform grid (10 rows and 5 columns) for the fixed photoquadrats, while for the random photoquadrats, 50 points were randomly placed.

Figure 8. Photographs of some of the corrals at El Coral, La Parguera.

Statistics

Three-way distance Permutational Multivariate Analyses of Variance (PERMANOVA) tests (Anderson 2001) were performed at each site (El Coral and Mario) to quadrat type (fixed and random) and date. 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 Coordinate Analysis (PCO) plots were produced to visualize differences in benthic assemblages between years.

Figure 9. Photographs of some of the corrals at Mario, La Parguera.

Results and Discussion

Collection

A total of 6,205 settlers were collected during the sampling season of 2018. Settlement was greater during this season than in 2017 (3,179). Lines were not stolen during this sampling collection, which could be the reason for the higher number of settlers. During this season, settlers were collected at two locations, Old Buoy and El Hoyo. The reason for this is because lines were stolen at Old Buoy in 2017. The settlement at El Hoyo was consistently lower than at Old Buoy during the sampling. The current was stronger at El Hoyo, compared to Old Buoy and many times during the collection, the lines were being dragged by the current. Dragging of the lines caused the plates to be deeper in the water column. The size of settlers collected during this season was much smaller than last settlement season. Most of the settlers collected during 2018 ranged in size from 0.4mm to 0.6mm. Large settlers (~1mm) were rare during the collection season.

Table 1. The total number of settlement plates and settlers (#) collected from June to October 2018.

Month	Settlers
June	300
July	622
August	1007
September	2826
October	1450
Total	**6205**

Grow-out

Survivorship of settlers was low during the grow-out (<10%). There were multiple problems during the rearing process. The settlers would lose spines and some would eventually die when water changes occurred. Water was then changed over a couple of days, which helped increase the survivorship and lower the stress of the settlers. Also, there was not sufficient amount of tank space for all the settlers. The growth of the settlers were stunted given the limited space. Settlers were transferred to the tanks outside to free up space in the inside tanks, but the survivorship was also low outside. The temperature in the outside tanks ranged greatly during the day (~10°C), which could have caused stress to the settlers and recruits. Another problem during 2018 summer was the low salinity in the outside tanks. Salinity was measured between 31-32 ppt in October and November. The low salinity measured could be due to the Orinoco River Plume entering along the south coast of Puerto Rico. Other sea urchin settlers (*Eucidaris tribuloides*), seem to not be affected by the change in water quality. On the other hand, settlers of *D. antillarum* are incredibly sensitive to any water quality. Therefore, a semi-closed system where temperature, oxygen and salinity are kept constant and maintained would be the better option for the rearing of settlers. Water changes are not necessary in a semi-closed system since there is a constant input of fresh seawater. The flow of the input needs to be low enough to maintain the water parameters.

Restocking

Before restocking, a visual assessment of *D. antillarum* populations was conducted at El Coral and Mario. The density of *D. antillarum* around the corrals was low (< 0.05 Ind m^{-2}), at both sites. At El Coral, there were some adult *D. antillarum* observed on shallow coral heads outside the area of study. Other sea urchins were more common, such as *Echinometra viridis* and *Tripnuestes ventricosus*. Initial assessments show that the most common algal type at both reefs was *Dictyota* spp. and thick turf algae with fine sediment (see Figure 3 and Figure 4). The turf alga and articulate calcareous algae (*Jania* spp.) were exceptionally high in areas where there were damselfishes.

Survivorship

Lab-reared *D. antillarum* were retrieved on August 31, 2019 from Media Luna and transferred to the corrals at El Coral and Mario. Twenty-three sea urchins were placed in each corral, giving a total of 138 restocked sea urchins at each site (total for study 276). The other 204 sea urchins were left at Media Luna because they were hidden in crevices and hard to remove. Monitoring took place during September 9th (one week), September 16th (two weeks), September 30th (one month), and October 28th, 2019 (two months). Tops were removed on all corrals after the second week of monitoring. This was due because the tops were getting tangled with octocorals and other benthic organisms.

As seen in Table 2 and 3, the retention of sea urchins in the corrals was higher at El Coral, than Mario. Most of the sea urchins escaped the corrals at Mario after the first month. There were at least couple (2-4) of threespot damselfish, *Stegastes planifrons*, in each corral at Mario. *S. planifrons* are one of the most aggressive damselfish in the Caribbean (Rampersad K, pers comm.). They fiercely guard and defend their territories and nip at potential predators or invaders. They are known to be more aggressive to conspecifics, like other herbivores, than to predators (Kapetanaki 2008). Many times, damselfish were noticed pecking at the spines of the reintroduced *D. antillarum*. Also, pieces of spines were commonly observed inside the corrals at Mario, however, urchin skeletons were never observed. Williams (1979) recorded threespot damselfish to be more aggressive to *D. antillarum*, compared to other sea urchins, thus directly affecting the distribution of sea urchins on the reef. The higher retention rates at El Coral could be due to the lower abundance of damselfish.

Table 2. The number of Diadema antillarum in the different corrals during the monitoring times at El Coral, La Parguera.

Date	Corral 1	Corral 2	Corral 3	Corral 4	Corral 5	Corral 6
8/31/2019	23	23	23	23	23	23
9/9/2019	13	11	24	8	17	20
9/16/2019	9	15	21	6	16	22
9/30/2019	8	8	7	6	12	18
10/28/2019	9	7	4	6	17	7

Table 3. The number of Diadema antillarum in the different corrals during the monitoring times at Mario, La Parguera.

Date	Corral 1	Corral 2	Corral 3	Corral 4	Corral 5	Corral 6
8/31/2019	23	23	23	23	23	23
9/9/2019	10	12	22	15	0	7
9/16/2019	2	5	8	9	0	3
9/30/2019	2	1	1	6	0	3
10/28/2019	0	1	0	3	0	2

The overall retention of sea urchins at El Coral was 36%, which is higher than the retention at Cayo Diablo and Los Lobos in Fajardo during the 2018 restocking. As seen in the 2018 restocking, we found many escaped sea urchins outside corrals after the one week of monitoring. As seen in both the Fajardo and La Parguera restocking, many of the escaped sea urchins were hidden in holes and crevices. Thus, making it difficult to remove them and place them back inside the corrals. The lower retention could also be due to predation. However, broken spines of *D. antillarum* were only recorded two times during the monitoring. Broken spines were observed in corral 3 at El Coral and corral 4 at Mario. These corrals had two to three resident threespot damselfish.

Benthic Cover

Control sites

As seen in Table 4 and 5, there was not a significant difference of benthic composition between quadrat types (p=0.26 and 0.24), and furthermore there was not a significant interaction between quadrat type and sampling time (p=0.15 and 0.72). Therefore, fixed and random quadrats were analyzed together to examine the benthic changes through time.

The dominant benthic substrate in the control stations at both La Parguera stations during the monitoring was fleshy (*Dictyota* spp., *Padina* spp., etc.) and articulate coralline algae (*Halimeda* spp., *Jania* spp., etc.) (Index 1-4). The benthic cover significantly changed over time at the control station at El Coral because the abundance of *Dictyota* spp. significantly increased by the second month by 460% (Fig. 10). The other abundant substrates at El Coral was *Halimeda* spp. and turf with sediment.

Table 4. The results of the two-way Permutational Analysis of Variance (PERMANOVA) to examine the changes in benthic cover between quadrat type (Qu), fixed and random, and sampling time (Ti) at the control site at El Coral, La Parguera.

Source	df	SS	MS	Pseudo-F	p value
Qu	1	2998.2	2998.2	2.5828	0.2617
Ti	1	5659.9	5659.9	10.618	0.004
QuxTi	1	1160.8	1160.8	2.1779	0.146

Table 5. The results of the two-way Permutational Analysis of Variance (PERMANOVA) to examine the changes in benthic cover between quadrat type (Qu), fixed and random, and time (Ti) at the control site at Mario, La Parguera.

Source	df	SS	MS	Pseudo-F	p value
Qu	1	2398.5	2398.5	3.3873	0.2435
Ti	1	1947.4	1947.4	1.0943	0.344
QuxTi	1	708.11	708.11	0.3979	0.715

Even though the benthic cover did not significantly changed over time at Mario (Table 5), there was also a significant increase in *Dictyota* spp. of 272% (one-way PERMANOVA, $p<0.0001$) (Fig. 11). The other common substrate at Mario was turf and turf with sediment. There was no grazing observed in the control area during the monitoring. This is evidenced by the significant increase of fleshy macroaglae. 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*.

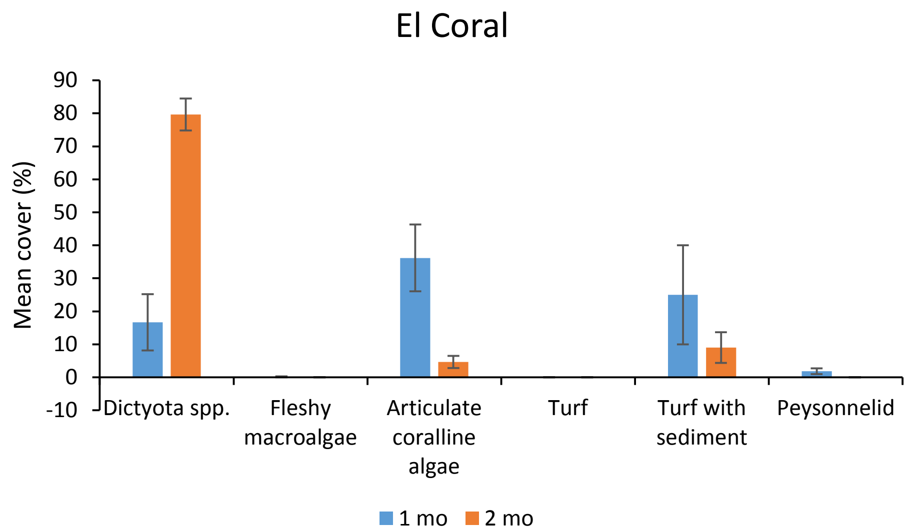

Figure 10. Mean benthic cover (%) at the control site at El Coral in La Parguera, Puerto Rico. The bars denote standard errors.

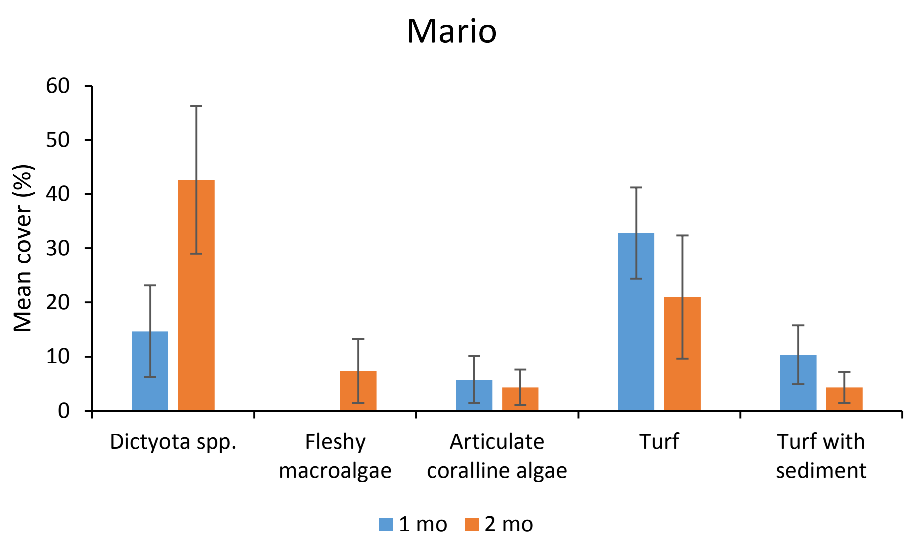

Figure 11. Mean benthic cover (%) at the control site at Mario in La Parguera, Puerto Rico. The bars denote standard errors.

El Coral and Mario

Pre-restocking

In the corrals at El Coral, the benthic substrate was characterized by high mean cover (± SE) of *Dictyota* spp. (44.6 ± 7.0% - 36.1 ± 5.4%) and turf with sediment (24.7 ± 5.5% - 21.0 ± 4.2%) in both the fixed and random quadrats, respectively (Fig. 12). Other fleshy macroalgae, such as *Padina* spp., and articulate coralline algae, *Halimeda* spp., were also a common abundance. Turf algal and crustose coralline algae (CCA) cover were low in fixed quadrats (5.0 ± 1.2% and 0.5 ± .1%) and random (5.9 ± 1.8% and 0%), respectively. The dominant substrate in the fixed and random corrals at Mario before the restocking, was turf algae with a mean cover of 25.5 ± 6.4% and 32.5 ± 7.1%, unidentified fleshy macroalgae (25.3 ± 3.7% and 8.4 ± 2.2%), and *Dictyota* spp. (17.2 ± 3.2% and 15.5 ± 5.2%) (Fig. 13). There was no CCA present in the corrals before restocking. Other substrates like turf with sediments and articulate coralline algae were present but in low numbers. The baseline cover in the corrals before restocking event were similar to those measured at the control sites (see above).

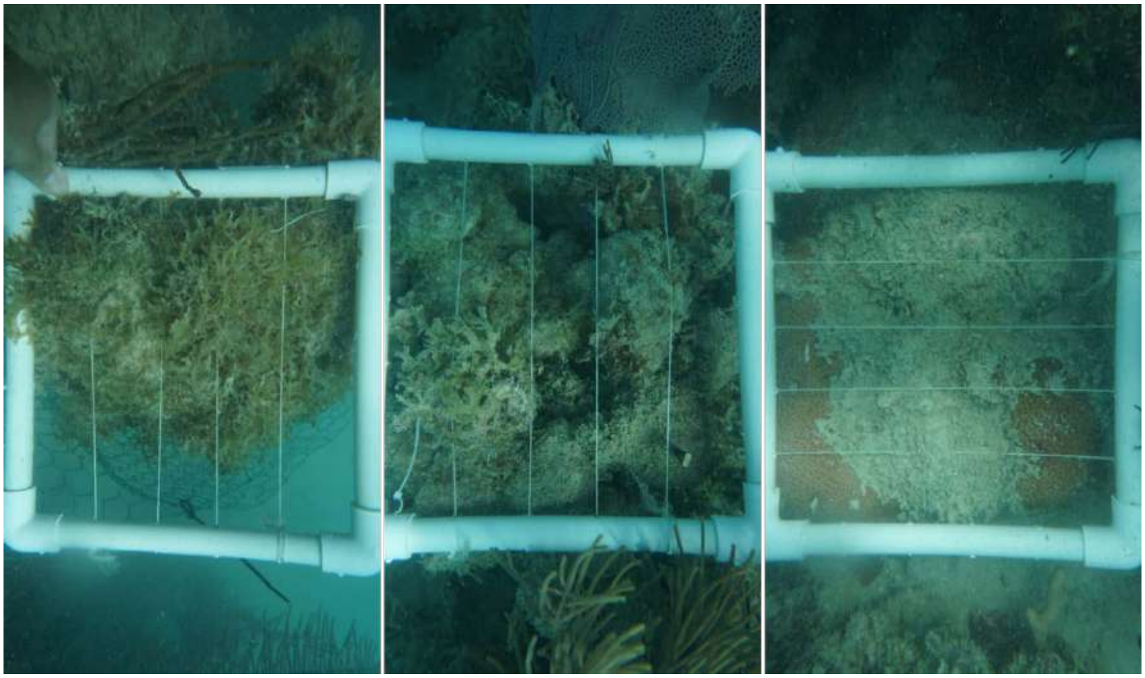

Figure 12. Photographs of permanent (left and middle) and random (right) quadrats inside corrals at El Coral, La Parguera, Puerto Rico.

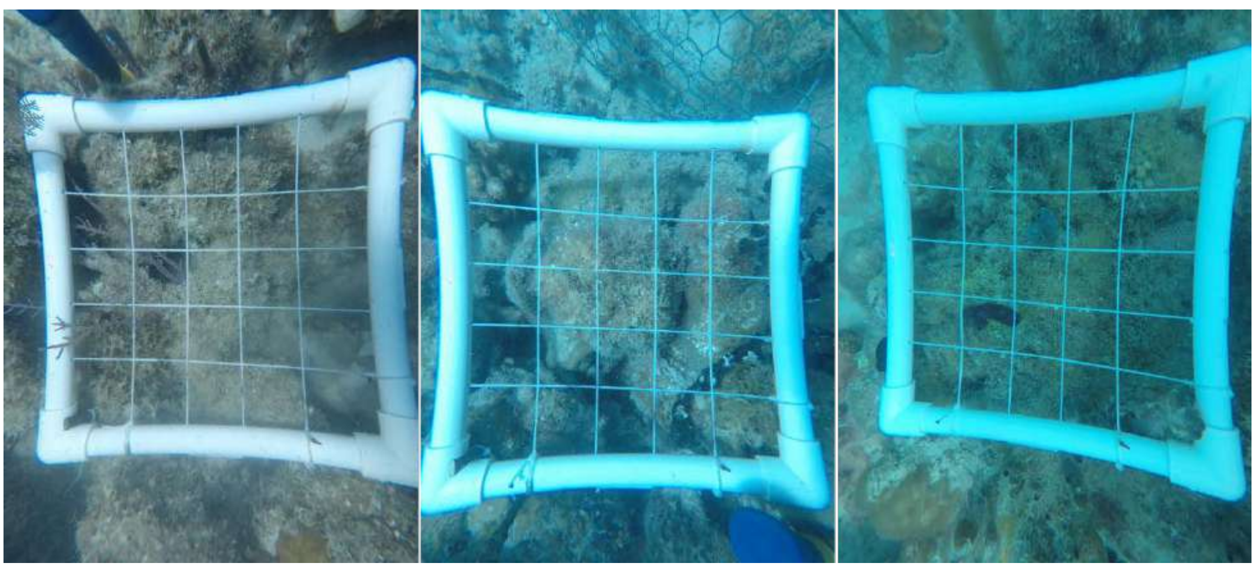

Figure 13. Photographs of permanent (left and middle) and random (right) quadrats inside corrals at Mario, La Parguera, Puerto Rico.

As reported for the baseline cover, the benthic substrate at both reefs are not optimal for the settlement and growth of corals. Fleshy macroalgae and turf with sediment can be detrimental for the settlement of post-larval corals and growth and survivorship of juvenile corals. Fleshy macroalgae, especially *Dictyota* spp. can retard growth rates of juvenile corals by outshading and abrasion (Box and Mumby 2007). The allochemicals produced by brown, fleshy macroalgae, can also introduce dangerous bacteria and inhibit coral larvae to settle (Morrow et al. 2017). Turf, which is short, filamentous and without sediment, will not affect the settlement and survivorship of corals (O'Brien and Scheibling 2018). However, quite often algal turf will accumulate sediments when there is a terrestrial source close by or there is constant resuspension of sediments. Birrel et al. (2005) found that coral settlement was significantly reduced when algal turf accumulated sediments. In the Florida Keys, turf with sediment significantly inhibit coral from settling by 10 to 13 fold, compared to turf algae alone (Speare et al. 2019). They also found that turf with sediment negatively affected juvenile corals. Given that both sites had relatively high cover of fleshy macroalgae and algal turf with sediments, we can conclude that before restocking sea urchins, the available and/or suitable substrate for corals to settle and survive was poor.

Table 6. The results of the 3-way Permutational Analysis of Variance test to examine the benthic composition change between corrals (Co), quadrat types (Qu), fixed and random, and sampling time (Ti) at El Coral, La Parguera.

Source	df	SS	MS	Pseudo-F	p value
Co	5	54705	10941	9.2291	**0.001**
Qu	1	8399.4	8399.4	9.9919	**0.015**
Ti	4	73311	18328	17.085	**0.001**
CoxQu	5	14899	2979.7	4.7615	**0.001**
CoxTi	20	23712	1185.6	1.1052	0.26
QuxTi	4	3362.3	840.57	0.78358	0.715
CoxQuxTi	20	12508	625.41	0.58301	0.996

Table 7. The results of the 3-way Permutational Analysis of Variance test to examine the benthic composition change between corrals (Co), quadrat types (Qu), fixed and random, and sampling time (Ti) at Mario, La Parguera.

Source	df	SS	MS	Pseudo-F	p value
Co	5	54909	10982	12.532	**0.001**
Qu	1	4971.8	4971.8	7.1968	**0.017**
Ti	4	30999	7749.6	8.847	**0.001**
CoxQu	5	14565	2912.9	3.957	**0.001**
CoxTi	20	17527	876.33	1.0004	0.482
QuxTi	4	2744.1	686.04	0.78318	0.722
CoxQuxTi	19	13965	734.99	0.83907	0.822

As seen in the PERMANOVA results (Table 6 and 7), the benthic composition inside the corrals was significantly different between corrals, quadrat types and sampling times. There was no significant interaction between these factors, except between corral and quadrat type. Given no significant interaction, benthic composition significantly changed through the sampling times, independent of quadrat type.

At both El Coral and Mario, the grazing effects of *D. antillarum* significantly changed the benthic composition inside the corrals and this was more pronounced through time at El Coral, and not so much at Mario (Tables 6 and 7). This would be expected since the grazing pressure was lower at Mario because most of the sea urchins escaped the corrals. Even after one week of the restocking, the grazing effects of *D. antillarum* were evident at El Coral (PERMANOVA, $t=3.5$, $p<0.0001$) and Mario (PERMANOVA, $t=3.9$, $p=<0.0001$). *Dictyota* cover was reduced between 52% and 54% at El Coral, and between 46% and 52% at Mario during the first week of restocking the sea urchins (Fig. 14-17). At El Coral, turf with sediment was significantly reduced between 37% and 45% (Fig. 14-17). Other fleshy macroalgae was significantly reduced between 87% and 95% at Mario.

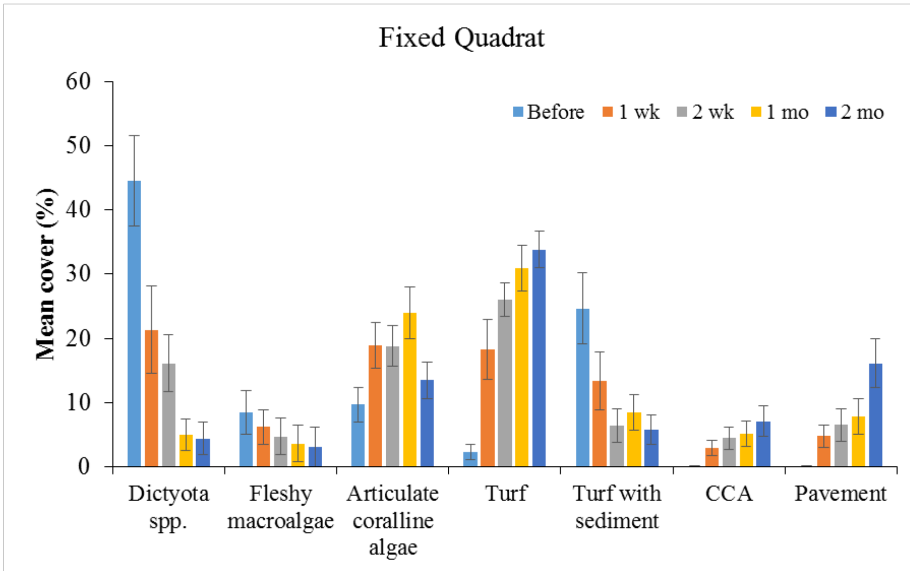

Figure 14. The mean cover (%) of the major benthic categories in the fixed quadrats before and after the restocking of Diadema antillarum at El Coral, La Parguera. The bars denote standard errors.

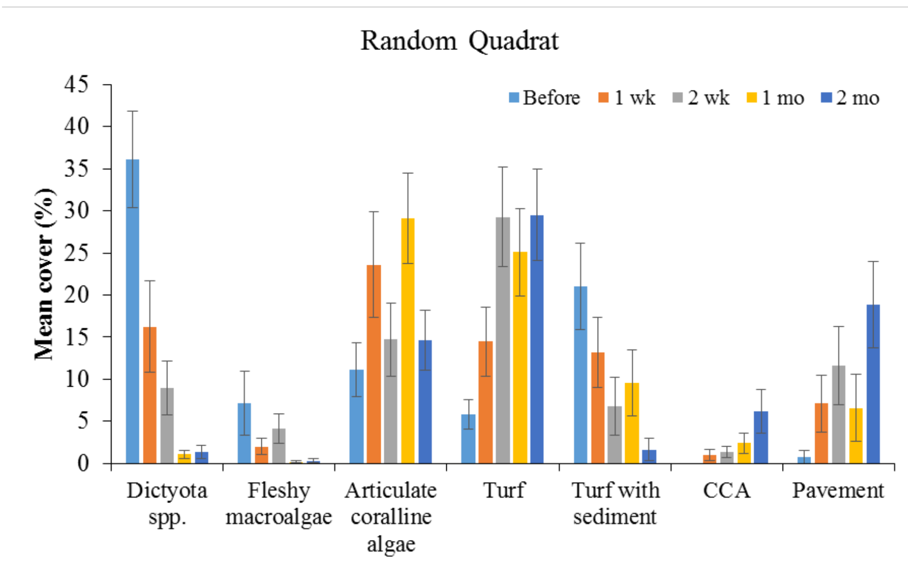

Figure 15. The mean cover (%) of the major benthic categories in the random quadrats before and after the restocking of Diadema antillarum at El Coral, La Parguera. The bars denote standard errors.

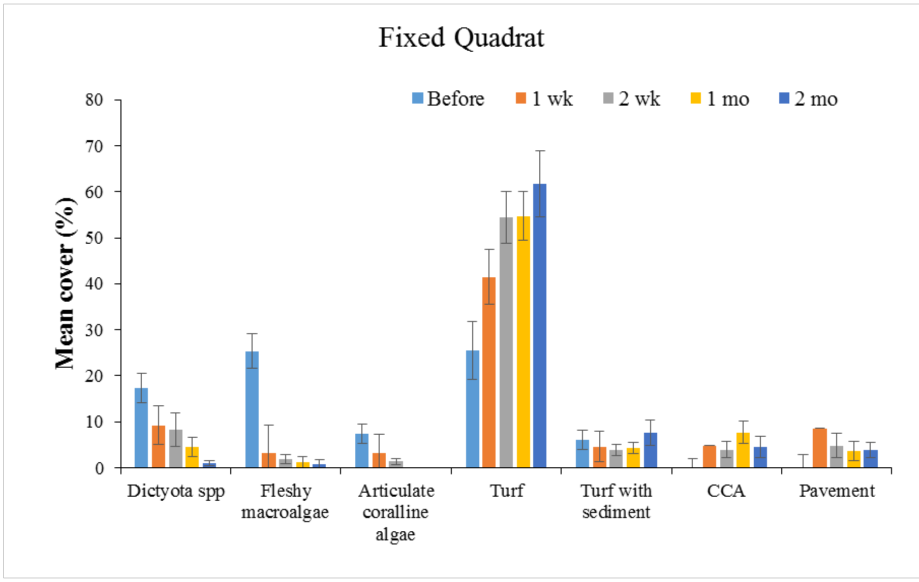

Figure 16. The mean cover (%) of the major benthic categories in the fixed quadrats before and after the restocking of Diadema antillarum at Mario, La Parguera. The bars denote standard errors.

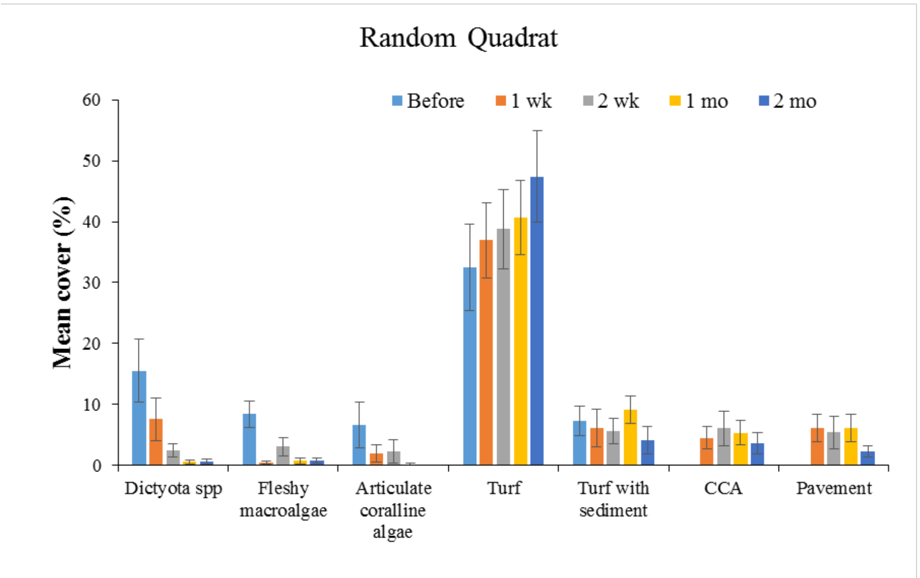

Figure 17. The mean cover (%) of the major benthic categories in the random quadrats before and after the restocking of Diadema antillarum at Mario, La Parguera. The bars denote standard errors.

After two months of restocking *D. antillarum* to El Coral, the reef substrate was characterized by more suitable substrates for coral settlement, such as small, filamentous turf, CCA, and clean substrate ("pavement") (Fig. 18). In the fixed quadrats, small, filamentous turf algae, and CCA and clean substrate ("pavement") increased through the sampling period by 93.3%, 98.2%, and 99.2%, respectively (Fig. 14 and 15). This trend was also observed in the random quadrats (Fig. 19). By the second month after restocking, *Dictyota* cover was reduced up to 90%, and turf with sediment, another unsuitable substrate, was reduced between 77% and 92%. Three small coral recruits were observed in corral 1 in two of the fixed quadrat. It is unknown if these recruits were newly settled or covered by macroalgae before the experiment. Longer monitoring of the grazing effects and alternative experiments need to be carried out to see if restocking sea urchins influences coral recruitment.

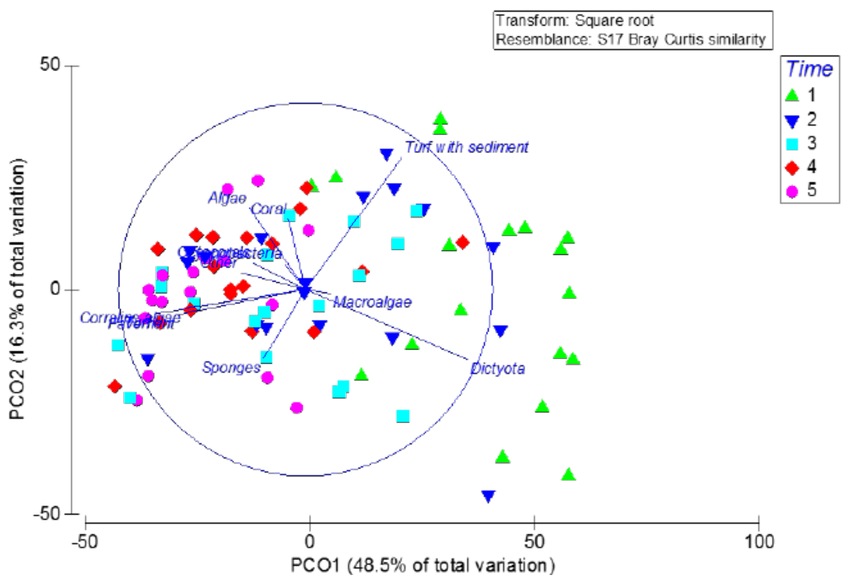

Figure 18. Principle coordinate analysis (PCO) of the change of benthic composition through time in the fixed quadrats at El Coral, La Parguera. 1=before restocking of Diadema antillarum, and after restocking 2= 1-week, 3=2-weeks, 4= 1 month, and 5= 2 months.

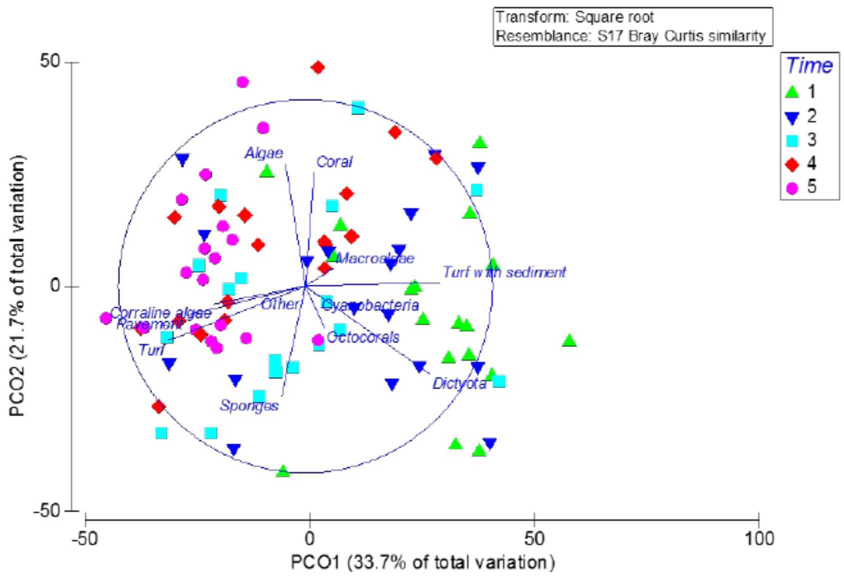

Figure 19. Principle coordinate analysis (PCO) of the change of benthic composition through time in the random quadrats at El Coral, La Parguera. 1=before restocking of Diadema antillarum, and after restocking 2= 1-week, 3=2-weeks, 4= 1 month, and 5= 2 months.

At Mario, most of the *D. antillarum* escaped after two months of the experiment and the grazing effects at Mario were not as pronounced as in El Coral. This can be seen by the size of the standard error bars in Figure 16 and 17, and the pair-wise PERMANOVA tests. Benthic composition did not significantly change after the one week of restocking (PERMANOVA, t=1.0, p=0.4). Even though the grazing was absent in most corrals, the impacts of restocking *D. antillarum* were still significant by the two month monitoring (PERMANOVA, t=1.7, p=0.02). During the two month monitoring, the substrate at Mario was still characterized by smaller, filamentous turf algae, in both the fixed and random quadrats (Fig. 20 and 21). And there was also cleaner substrate quantified in the fixed quadrats after two months of restocking (Fig. 11 and 12).

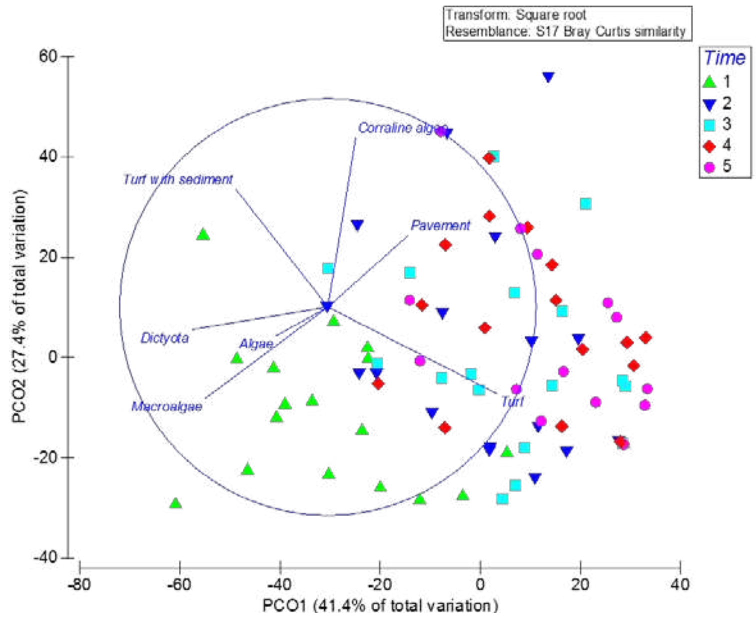

Figure 20. Principle coordinate analysis (PCO) of the change of benthic composition through time in the fixed quadrats at Mario, La Parguera. 1=before restocking of Diadema antillarum, and after restocking 2= 1-week, 3=2-weeks, 4= 1 month, and 5= 2 months.

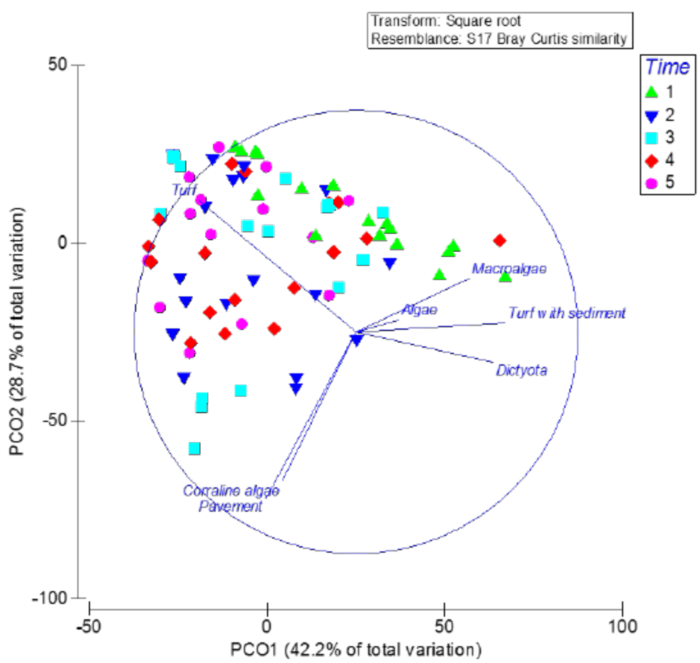

Figure 21. Principle coordinate analysis (PCO) of the change of benthic composition through time in the fixed quadrats at Mario, La Parguera. 1=before restocking of Diadema antillarum, and after restocking 2= 1-week, 3=2-weeks, 4= 1 month, and 5= 2 months.

Conclusions and recommendations

- *D. antillarum* settlers were more sensitive to water quality changes than other species of sea urchin. *D. antillarum* settlers were greatly impacted by the variations of salinity, and temperature.
- Given the results of 2018 and 2019 restocking, it can be concluded that the restoration of *D. antillarum* significantly changes the benthic composition on coral reefs in Puerto Rico.
- *D. antillarum* effectively consumes and controls the abundance of most macroalgae, especially *Dictyota* spp. They also significantly reduce the abundance of turf with sediment, which could have detrimental effects on corals and their settlement.
- On the other hand, the restoration of *D. antillarum* enhances a more suitable reef substrate, by increasing clean substrate and the cover of crustose coralline algae (CCA).
- The grazing impacts of *D. antillarum* has long lasting effects (>2months) even after they are removed or escape the experimental area.
- Damselfish, especially the threespot damselfish, could be influencing the retention of *D. antillarum* on the coral reefs. Retention might be increased if restoration occurs on coral reefs with a lower abundance of damselfishes. This topic needs to me further studied.
- Given the results of this study, the restocking of *D. antillarum* might be an effective mitigation tool in the reductions of algal cover, especially fleshy macroalgae and turf with sediment.

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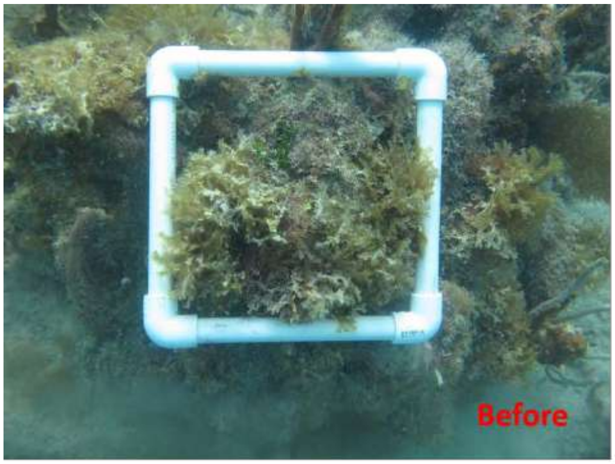
Indices

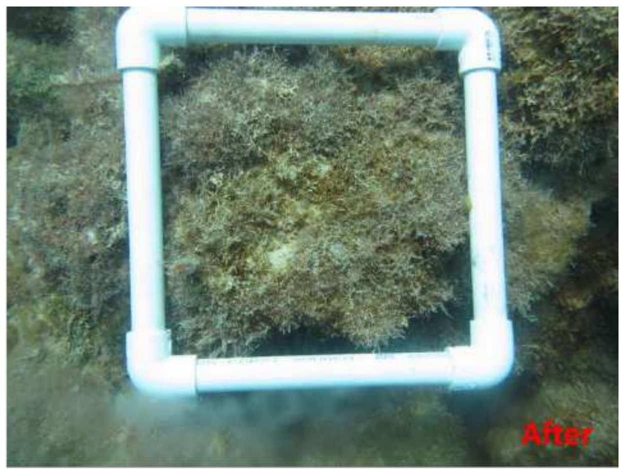
Index 1 Photographs of permanent quadrats at the control station before and one month after the restocking of Diadema antillarum at El Coral, La Parguera, Puerto Rico.

Index 2 Photographs of random quadrats at the control station before and one month after the restocking of Diadema antillarum at El Coral, La Parguera, Puerto Rico.

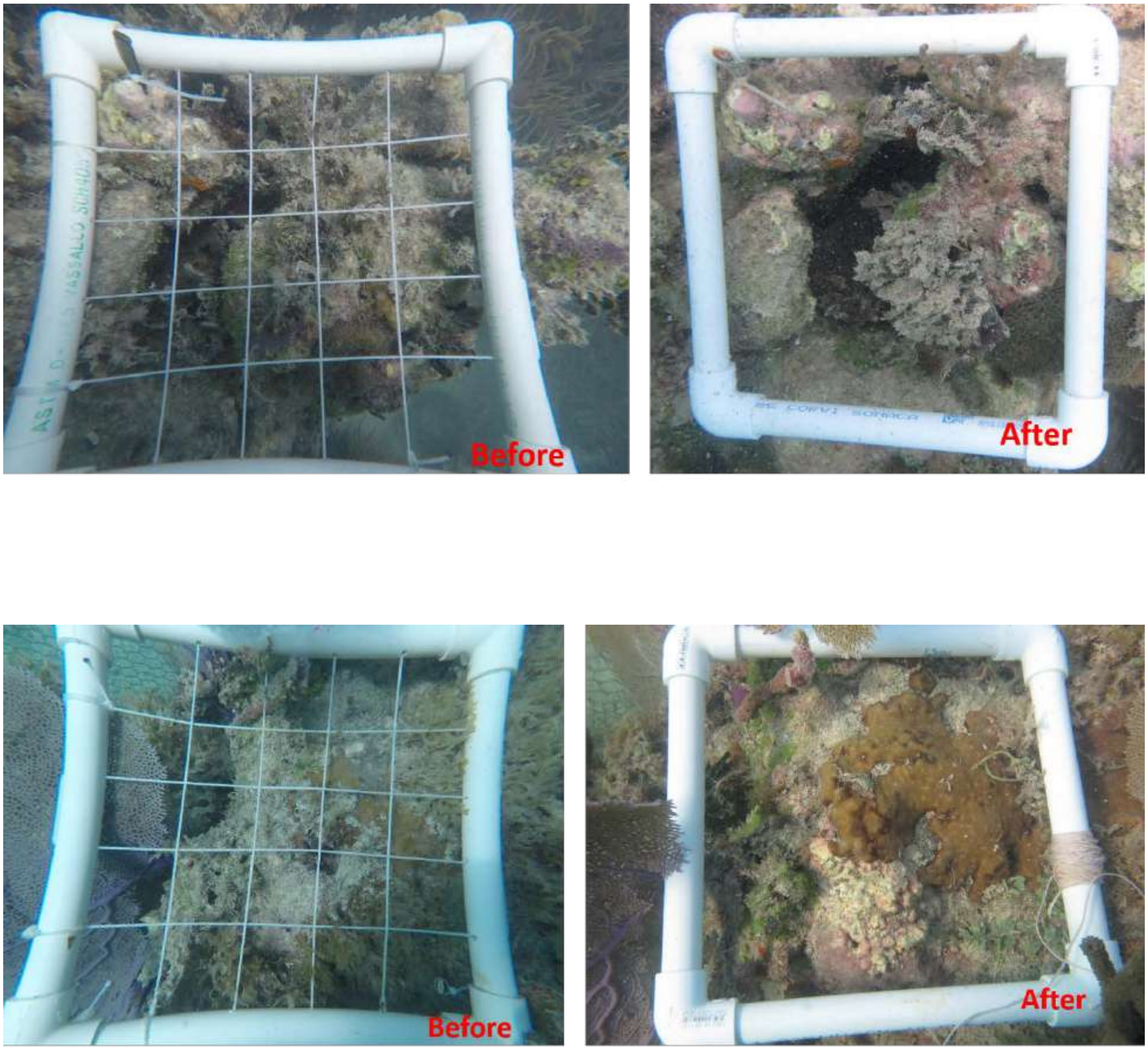
Index 3. Photographs of permanent quadrats at the control station before and one month after the restocking of Diadema antillarum at Mario, La Parguera, Puerto Rico.



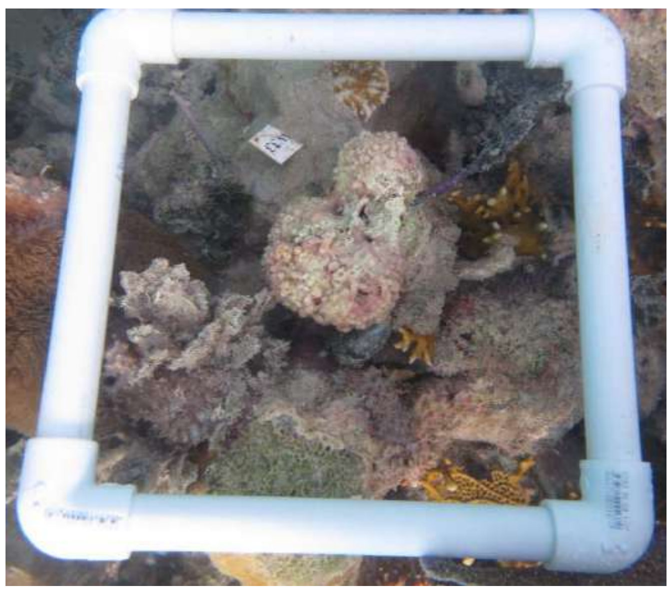
Index 4. Photographs of random quadrats at the control station before and one month after the restocking of Diadema antillarum at Mario, La Parguera, Puerto Rico.

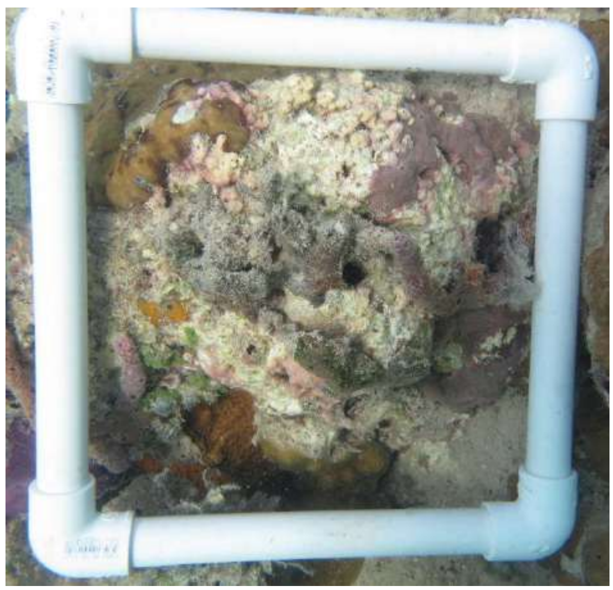


Index 5. Photographs of permanent quadrats before and two months after the restocking of Diadema antillarum at El Coral, La Parguera, Puerto Rico.

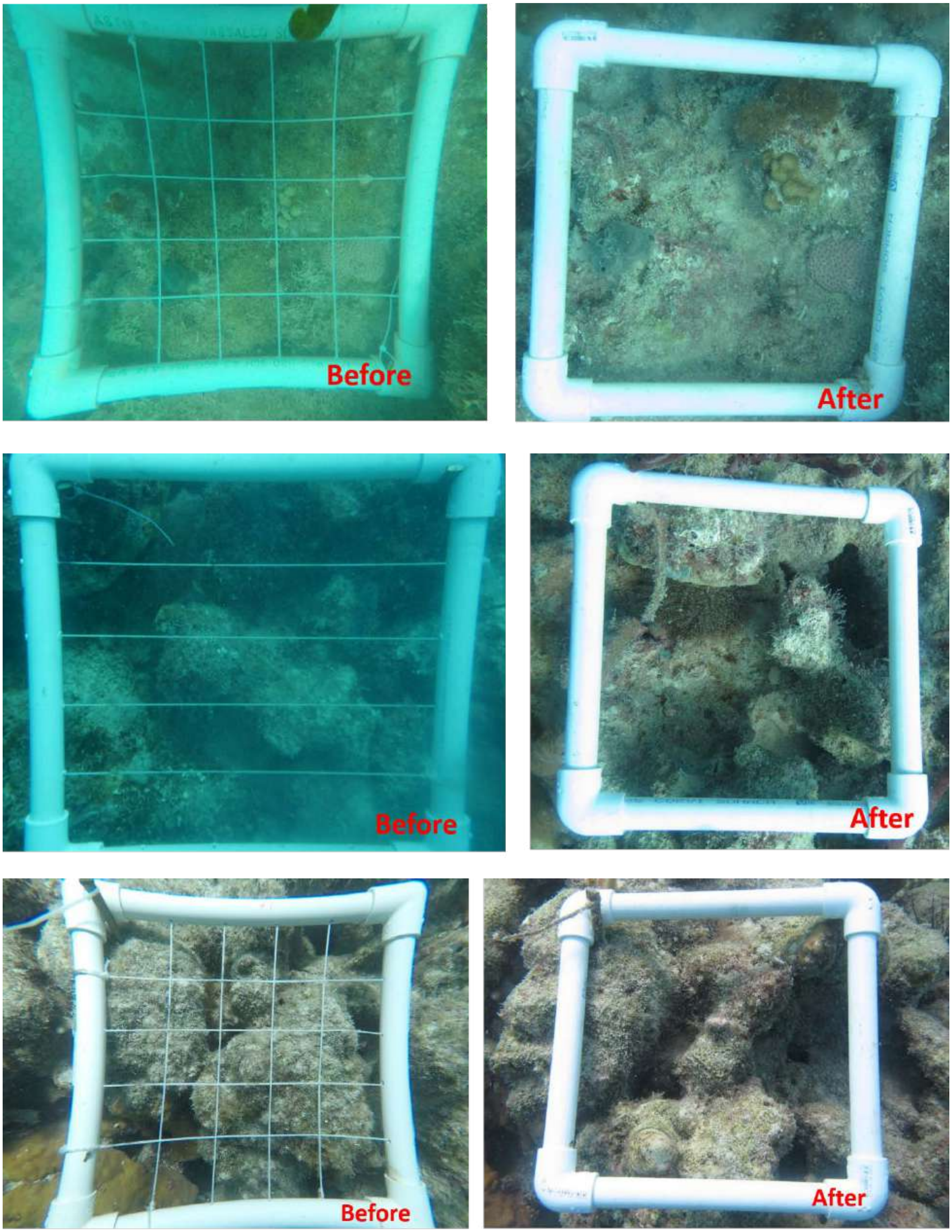


Index 6. Photographs of the grazing effects of Diadema antillarum two months after restocking in the corrals at El Coral, La Parguera, Puerto Rico. These are individual photographs taken of areas where the substrate has been grazed by Diadema antillarum.

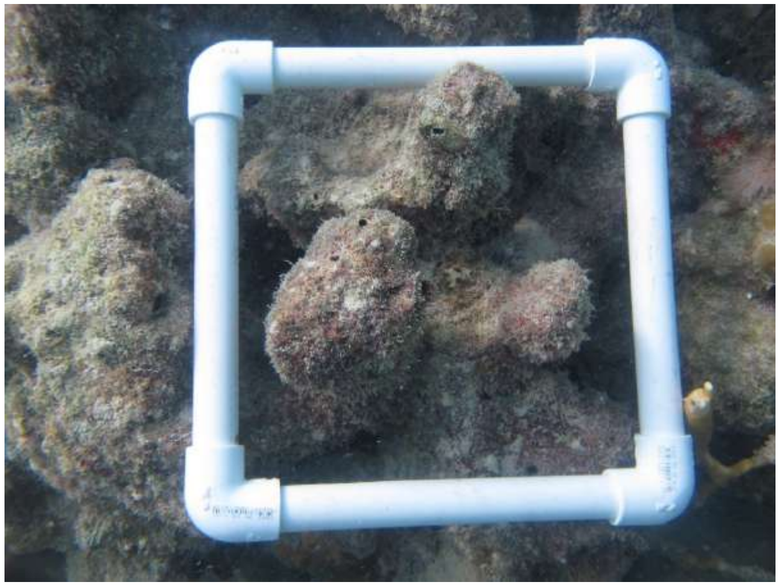


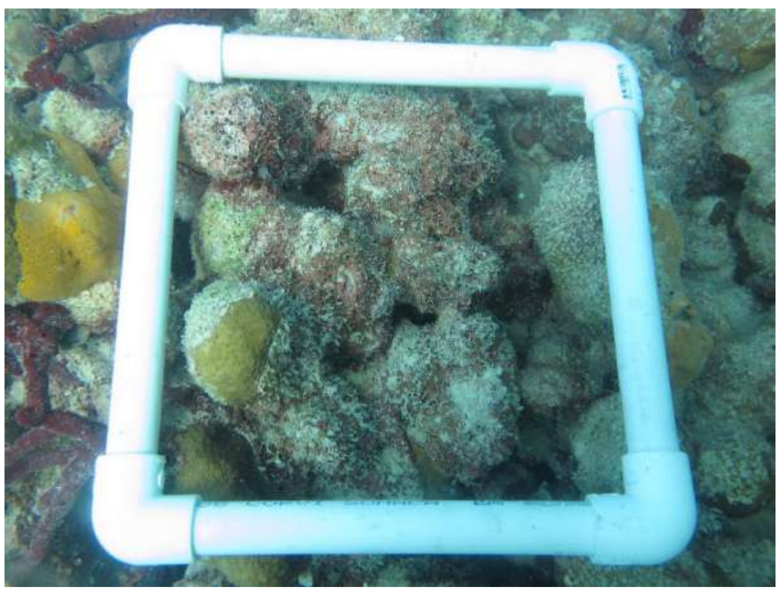


Index 7. Photographs of permanent quadrats before and two months after the restocking of Diadema antillarum at Mario, La Parguera, Puerto Rico.



Index 8. Photographs of the grazing effects of Diadema antillarum two months after restocking in the corrals at Mario, La Parguera, Puerto Rico. These are individual photographs taken of areas where the substrate has been grazed by Diadema antillarum.





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