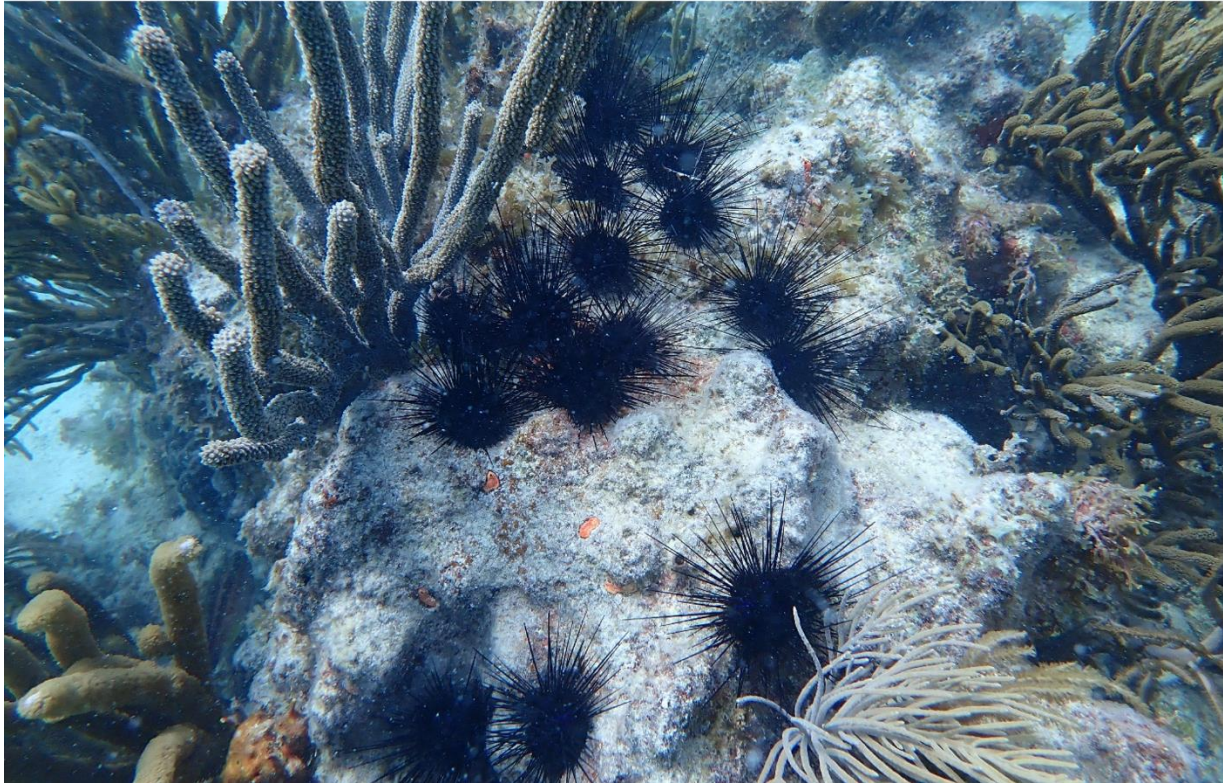


Restocking of keystone herbivore species, *Diadema antillarum*, to reduce algal overgrowth (Phase II)



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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. On the east coast of Puerto Rico, the corals are competing for space with another algae competitor, *Ramicrusta* spp. Fleshy macroalgae, turf with sediment and *Ramicrusta* are detractors 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 out shading 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. *Ramicrusta* is detrimental to the coral reef because it can overgrow, and smother live coral tissue (Eckrich and Engel 2013). The objective of this project was to increase herbivory rates on two coral reefs in Fajardo, Cayo Largo and Palomino to decrease the algal abundance, specifically *Ramicrusta* spp, fleshy macroalgae and turf with sediment.

On November 12, 2021, 500 lab-reared *Diadema antillarum* were transported by cargo van to Puerto del Rey Marina in Ceiba, Puerto Rico. At each reef, 20 sea urchins were placed in three experimental corrals, and the rest were released onto the reef in groups between 30 to 40 individuals. The change in benthic composition was monitored with three permanent 10 cm² photoquadrats in experimental corrals, procedural control (corrals without *D. antillarum*) and the control plot outside the corrals. Sea urchins and the benthic substrate were monitored before the

restocking and one week, two weeks, one month, two months and three months after reintroduction. *D. antillarum* densities were measured along three random 20m² belt transects before and two-months after the restocking of sea urchins. Corrals and rebars were removed from each reef after the three-month monitoring.

Cayo Largo and Palomino were characterized by high abundance of *Dictyota* spp. and *Ramicrusta* spp. Before the sea urchin restocking, the benthic composition did not significantly differ inside the experimental and control treatments. However, the significance in benthic change throughout the monitoring period was dependent on whether *D. antillarum* were present inside the corrals at Cayo Largo and Palomino. There was a significant herbivory effect on the change of benthic composition at both sites. There was a significant difference in benthic assemblage inside the experimental corrals after just two-weeks and one month after restocking the sea urchins at Cayo Largo and Palomino, respectively. After two weeks, *D. antillarum* almost completely removed *Dictyota* at Cayo Largo, reducing the cover by 93 % and after one month, *Ramicrusta* was reduced by 90 % at the end of the monitoring period. By two months at Cayo Largo, clean substrate increased by two orders of magnitude, from 4.89 ± 4.89 % to 84 ± 7.15 %.

Grazing effects of *D. antillarum* were slower at Palomino compared to Cayo Largo. This could be due to the physical environment at Palomino, as it is more exposed and vulnerable to swells and wave action. *D. antillarum* significantly reduced *Ramicrusta* by 38 % in corral 1 and 3 after two months (Index 6). In addition, *D. antillarum* eliminated an encrusting sponge, *Dictyonella funicularis*, in corral 3 also after two months. Clean substrate increased by two orders of magnitude inside the corrals with *D. antillarum*. In situ observations, confirmed grazing marks on *D. funicularis*. This is the first report of *D. antillarum* consuming this species of sponge.

In conclusion, the restocking of *D. antillarum* contributed to positive changes in benthic cover, as these sea urchins significantly reduced the algal cover on both coral reefs in Fajardo. This study, along with previous *D. antillarum* restoration efforts, prove that this sea urchin is effective in reducing the algal cover on coral reefs, especially *Ramicrusta* spp. and other peysonnelids. The restocking of *D. antillarum*, and possibly other species of sea urchins (*Echinometra viridis* and *Tripneustes ventricosus*), should be a focus in other restoration programs.

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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). More recently, coral reefs in Puerto Rico have been significantly impacted by two other threats, Stony Coral Tissue Loss Disease (SCTLD) and the proliferation of *Ramicrusta* spp. *Ramicrusta* spp., a dangerous encrusting peyssonnelid, has dominated the cover on the coral reefs on the east coast, and is slowly spreading to the south and west coasts of Puerto Rico. This alga is a threat to slow-growing sessile-benthic organisms, such as corals, as it can overgrow live tissue. *Ramicrusta* also reduces the area of suitable substrate for the settlement of coral and other benthic organisms. Parrotfish and other herbivorous fishes have not been observed consuming *Ramicrusta* spp. (Williams 2021). However, a keystone herbivore, *Diadema antillarum*, consumes and significantly reduces *Ramicrusta* (Williams 2021). However, the overall abundance of *D. antillarum* is low and patchy, usually limited to shallow-water reefs in Puerto Rico.

Before their mass mortality in 1983, *D. antillarum* was common and abundant (13-18 ind m⁻²) on both shallow and deep (>10m) coral reefs in Puerto Rico (Bauer 1980; Vicente and Goenaga 1984). *D. antillarum* played an essential role in 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) and productivity (Williams 1990) on the coral reefs. *D. antillarum* 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 long absence of *D. antillarum* on many reefs has not only influenced the benthic algal productivity of coral reef communities, but it also has impinged on other reef processes (i.e., coral recruitment) (Hughes et al. 1987).

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 to 2013. In addition, populations were dominated by medium to large (5-9 cm test diameter) individuals and were concentrated at shallower (< 5m), more structurally complex reefs. It has been proposed that either larval 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, 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.

Many Caribbean coral reefs have lost their capacity to recover from recurrent disturbances and have undergone long-term phase shifts (Hughes et al. 2003). The lack of herbivores on corals reefs is one of the reasons for the lack of recovery, which has led to a shift from coral to fleshy macroalgal and/or peyssonnelids on many of these coral reefs (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, increase 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. Restoring herbivores through conservation efforts such as protection and/or restocking, is a mitigation approach in reducing algal cover on coral reefs.

Since 2014, *D. antillarum* settlers have been collected and lab-reared in Puerto Rico. In 2016, the first major restocking of lab-reared *D. antillarum*, 343 young adults (3-4 cm in test diameter) were taken to the backreef of Media Luna in La Parguera (Williams 2016). In 2018 and 2019, 480 lab-reared *D. antillarum* were transferred to two reefs in Fajardo and 276 were transferred to Enrique's backreef in La Parguera. Significant changes in the benthic structure were observed during the first week after the restocking in all restoration projects. Significant reductions of fleshy macroalgae (*Dictyota* spp.) and thick turf algal/sediment mats (TAS), both unsuitable substrates (e.g., coral settlement), contributed to this change. Also, restocked *D. antillarum* significantly reduced the cover of encrusting red algae, *Ramicrosta* spp. By the end of the studies in 2018 and 2019, the abundance of fleshy macroalgae decreased by a mean of 77% (max of 100%) and *Ramicrosta* and TAS by 53% (max 71%) and 56% (max 100%), respectively. Clean substrate ("pavement"), crustose coralline algae (CCA), and filamentous turf algae increased between one to two orders of magnitude (Williams 2021).

The goal of this project was to increase the population of *D. antillarum* at two coral reefs in Fajardo, Cayo Largo and Palomino. In addition, this project aimed to record the herbivory effects of restocking *D. antillarum* on the abundance of nuisance algae such as fleshy macroalgae (i.e., *Dictyota* spp.) and *Ramicrosta*.

Methods

Settler grow-out

Settlers (Fig. 1) were picked off each settlement plates and counted at the Marine Science Department of the University of Puerto Rico, Mayagüez. We modified the receiving tanks for the small settlers to improve settler survivorship. Settlers were transferred to 10, 15-gallon aquariums (Fig. 2). Water was filtered through a cartridge filter

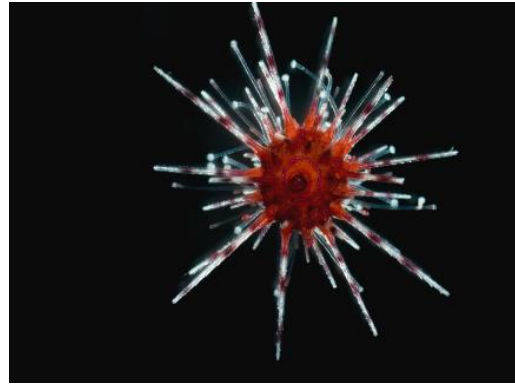


Figure 1 *Diadema antillarum* settler (~1 mm test diameter).

and 200-micron socks to remove sediments and a UV light to kill any bacteria or virus. Small settlers were given crustose coralline algae, filamentous algae and *Ulva* sp. for food. *Ulva* was washed before with freshwater to eliminate any potential predators from entering the tanks. Settlers



Figure 2 Seawater system for the newly collected settlers of *Diadema antillarum*.

were in these aquariums for two to three months until they reached a size greater than 2mm in test diameter and considered a “recruit”. Recruits were then placed in another seawater system containing two 45-gallon and one 65-gallon aquarium (Fig. 3). Seawater in this system was filtered through socks and UV sterilization.

Urchins in this system were primarily fed *Ulva* sp. Settlers at this size are sensitive to changes in water quality and parameters. Therefore, both seawater systems were connected to a semi-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). Temperature, salinity and pH in both systems were kept between 28-29 °C , 34-35 ppt and 8.0, respectively. All aquariums were cleaned weekly.



Figure 3 Seawater system containing the two 45-gallon and 60-gallon aquarium for the *Diadema antillarum* settlers.

replaced and cleaned once a week and raceways were cleaned once every week. Algae were collected and placed in each raceway every three to four days. Algal species, *Acanthophora*, *Chaetomorpha*, *Padina*, *Styopodium*, *Ulva sp.* 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

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 once a week and fresh saltwater entering the system is filtered through a sock. The sock was



Figure 4 Outdoor seawater tank system for the larger *Diadema antillarum*.

reached a test size of at least 3-4 cm in test diameter. Grow-out process took about a year.

Corral installation

On November 9, 2021, six corrals were installed at Cayo Largo, and another six were installed at Palomino reef in the Northeast Ecological Corridor Marine Extension, Fajardo, Puerto Rico (Table 1, Fig. 5).

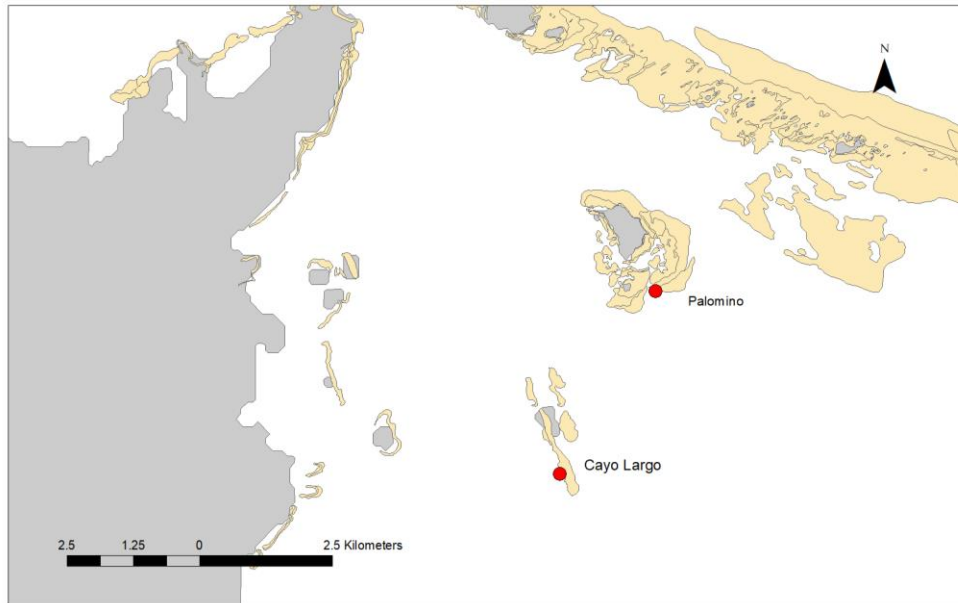


Figure 5 Map of the restocking sites, Cayo Largo and Palomino in Fajardo, Puerto Rico.

Table 1 The coordinates of the corrals at the two restocking sites, Cayo Largo and Palomino in Fajardo, Puerto Rico.

Site	Corral #	Latitude	Longitude
Cayo Largo	1	18.30732	-65.57947
	2	18.30730	-65.57944
	3	18.30729	-65.57942
	4	18.30728	-65.57936
	5	18.30727	-65.57946
	6	18.30722	-65.57950
Palomino	1	18.33838	-65.56326
	2	18.33831	-65.56322
	3	18.33817	-65.56316
	4	18.33815	-65.56329
	5	18.33823	-65.56319
	6	18.33819	-65.56318

Cayo Largo is a long, partially submerged fringing reef located southwest of Palomino reef (Fig. 5). Corrals were installed on the leeward side of the reef on the most southern part at 4-5 meters of depth. At this depth the benthic habitat was characterized by mostly dead *Orbicella annularis* and *O. faveolata* colonies. Patches of sand separated the individual coral colonies, making it easier to install the corrals. *Thalassia testudinum* plains bordered the deeper parts of this reef, while in shallower waters (<2 m) sand become less prominent and the hard substrate was characterized by dead *O. annularis* and *Acropora palmata*. *D. antillarum* was absent along the three, 20 m² belt transects conducted in the area of restocking. Also, we did not observe any triggerfish.

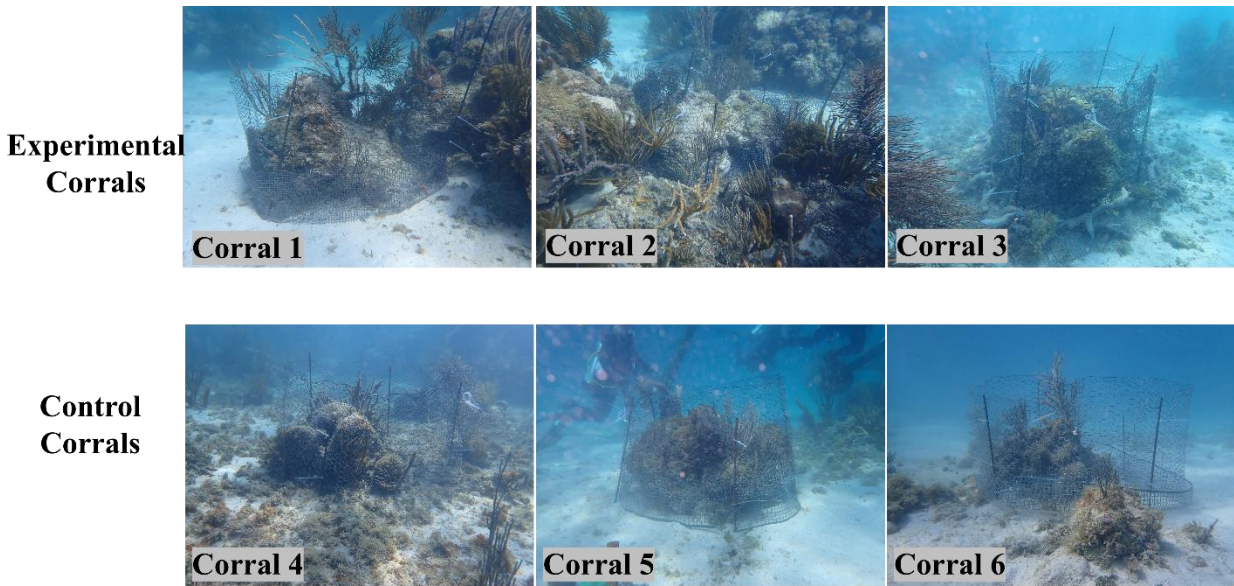


Figure 6 Pictures of each corral at Cayo Largo in Fajardo, Puerto Rico.

Palomino reef is a larger, fringing reef located within the Arrecifes de la Cordillera in Fajardo. This site is more exposed to wave action and the north swells. The corrals were installed on the southeastern side of the island at a site called Sand Slide at depths between 4-6 meters. This site is the location of Sea Ventures Dive Company's in situ coral nursery. The coral reef habitat at this site is quite different from Cayo Largo as it is comprised of large spur and groove formations running northeast to southwest. Three of the experimental corrals were installed on top of the spur formation and the other three were installed at the base of the spurs. Two adult *D. antillarum* were observed along the three, 20 m² belt transects (0.03 ± 0.02 ind m⁻²) carried out on each of the spur and groove formations. We did occasionally see ocean triggerfish (*Canthidermis sufflamen*) in the area of the corrals.

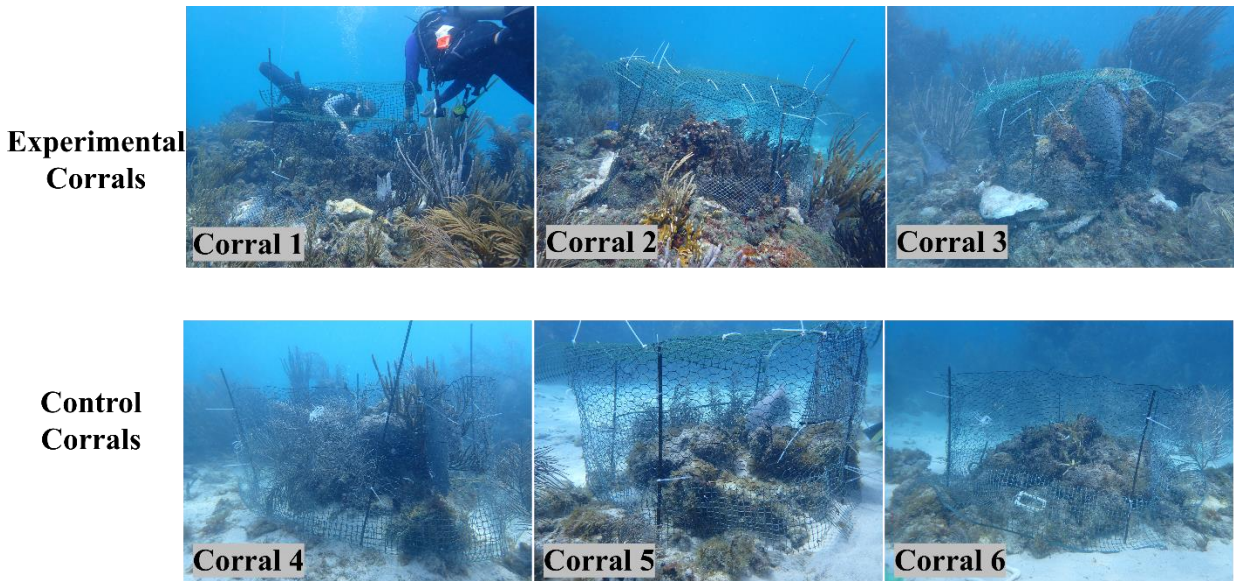


Figure 7 Pictures of each corral at Palomino in Fajardo, Puerto Rico.

At each site, there were a total of three experimental plots (corrals with *D. antillarum*) and three procedural controls, which were corrals without any *D. antillarum* (Fig. 6, 7). In addition, there was a control plot that was monitored outside the corrals. Corrals were installed at each reef from 4 to 5 meters of depth at Cayo Coral and 4 to 6 meters of depth at Palomino. Corrals were held into place with rebars. The diameter of each corral was approximately 1m². Corrals were made of metal chicken wire coated with plastic coating with a 1-inch diameter mesh size (Fig. 6, 7). Additional plastic netting was attached to the bottom of the corral to mold to the reef and act as a skirt. Corrals were fully enclosed (plastic netting for the tops) for the first month. The tops were removed after the one month monitoring.

To monitor the change in benthic substrate cover before and after *D. antillarum* restoration, photographs of the permanent quadrats were taken one week, two weeks, one month and two months after the restocking of *D. antillarum*. Nails were used to mark the position of permanent

quadrats (Fig. 8). *D. antillarum* were counted inside and outside the corrals during each monitoring. 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, 25 points were placed in a uniform grid (5 rows and 5 columns) for the permanent photoquadrats. Organisms were identified down to species and algae was discriminated to the lowest possible taxonomic level.



Figure 8 Photographs of monitoring the permanent quadrats.

Data analysis

Two, two-way distance Permutational Multivariate Analyses of Variance (PERMANOVA) tests (Anderson 2001) were performed at each site (Cayo Largo and Palomino) to assess the differences in benthic composition between time and plots (experimental: 1-3 vs controls: 4-6, outside). All data was square-root transformed. PERMANOVA procedures were based on Bray-Curtis similarity measures, and p-values were obtained using 9,999 permutations of the residuals under a reduced model. The *D. antillarum* abundance varied inside the experimental plots during the monitoring, therefore possibly affecting the change in benthic composition. The abundance of *D. antillarum* recorded was included as a covariable in each

PERMANOVA analyses. SIMPER tests were run to identify the contribution of benthic categories to the overall differences between monitoring time with the random and permanent quadrats and at the different sites.

Results and Discussion

Sea urchin transportation

On November 12, 2021, 500 lab-reared *Diadema antillarum* were transported by cargo van to Puerto del Rey Marina in Ceiba, Puerto Rico. Fifty sea urchins were placed in a blue bin (10 in total) and each bin was supplied with constant flow of air (Fig. 9). The urchins were loaded into the van in the early morning (6 AM AST) at Magueyes Island, La Parguera in order to arrive in Ceiba by late morning.



Figure 9 Picture of transportation of *Diadema antillarum* from La Parguera, Lajas to Puerto del Rey Marina in Ceiba, Puerto Rico.

D. antillarum were first restocked to Palomino reef and then Cayo Largo. Twenty sea urchins were placed in each of the experimental corrals (corral 1, 2 and 3) at Cayo Largo and Palomino. The tops of the corrals were removed directly after the one-month monitoring. The removal of the tops was due to the low abundance of algae in the corrals after one month. The rest of the urchins (190 sea urchins) were placed in groups of 30-50 individuals and were scattered around the proximity of the corrals (see Fig. 10, Table 2).

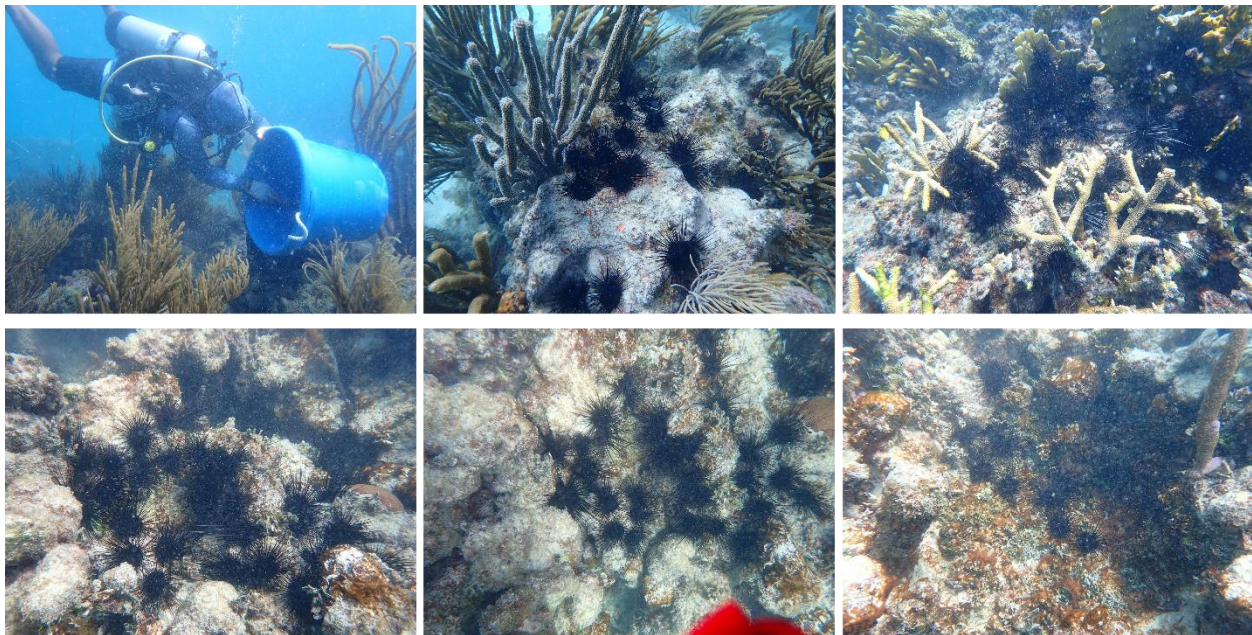


Figure 10 Top three pictures are of newly released *Diadema antillarum* at Palomino, and at Cayo Largo reef (bottom) in Fajardo, Puerto Rico.

The variation in *D. antillarum* retention in the corrals had no significant effect on the herbivory effects of the experiment (Index 1, 2). Overall, there was a low number of *D. antillarum* that escaped during the first month, with the exception of corral 2 at Palomino. As seen in Olmeda-Saldaña et al. (2021) experiment, a *D. antillarum* density of 5 ind m⁻² can have significant impacts on the benthic composition, especially on algal cover through time. At the end of the experiment, the density of sea urchins inside Cayo Largo's corrals was close to pre-mass mortality densities. While at Palomino, the urchin retention inside the corrals was much lower than at Cayo Coral, but

closer to what we would expect (or hope) to see on present day coral reefs, densities ranging between 2 to 5 ind m⁻².

Overall, there was a higher retention rate of *D. antillarum* at Cayo Largo than at Palomino reef. However, *D. antillarum* numbers estimated at Palomino may be conservative, as it was difficult to find the urchins because they were many times well hidden under coral heads and inside crevices. Ideally, *D. antillarum* should be surveyed during the night, as this would give a better estimation of abundance. We did not observe any mortality except during the last monitoring (2-mo) at Palomino. A skeleton and pile of spines was observed in corral 1. Ocean triggerfish were regularly observed at Palomino. They are a major predator of *D. antillarum*. Also, some of the *D. antillarum* at Palomino had shorten spines, which is an indication of damselfish harassment. Another possible reason why the *D. antillarum* were well hidden at Palomino is the wave energy. Palomino is more exposed to wave energy and the north swells. Through restocking, *D. antillarum* densities (mean \pm se) increased from one order of magnitude at Palomino (0.03 ± 0.02 ind m⁻² to 0.73 ± 0.13 ind m⁻²) to two orders of magnitude at Cayo Largo (0 ind m⁻² to 0.73 ± 0.46 ind m⁻²).

Table 2 The abundance of lab-reared *Diadema antillarum* in each of the experimental corrals (1, 2 and 3), and outside the corrals during the different monitoring periods at Cayo Largo and Palomino in Fajardo, Puerto Rico. The retention rate is the total amount of lab-reared urchins counted divided by the initial number of urchins released on each reef.

Site	Site	Corral 1	Corral 2	Corral 3	Outside	Total	Retention Rate
Cayo Largo	11/12/2021	20	20	20	190	250	100
	11/19/2021	20	20	20	190	250	100
	11/30/2021	16	20	16	125	177	70.8
	12/23/2021	14	20	14	135	183	73.2
	1/13/2021	12	12	16	146	186	74.4
Palomino	11/12/2021	20	20	20	190	250	100
	11/19/2021	20	15	20	193	248	99.2
	11/30/2021	20	0	19	110	149	59.6
	12/23/2021	19	0	20	73	112	44.8
	1/13/2021	1	0	8	64	73	29.2

Benthic composition

Before restocking sea urchins

Cayo Largo

As seen in Table 3, the dominant substrate at Cayo Largo before the restocking of sea urchins was benthic algae. *Dictyota* spp., followed by *Ramicrusta* spp. were the main contributors of the algal assemblage at this reef. The cover of these two substrates didn't differ between the corrals (Index 3). Clean substrate was not present in the procedural controls and the control outside the corrals. Very little amount (mean \pm SE, 4.89 ± 4.87 %) of clean substrate was observed inside the experimental corrals.

Table 3 Mean percent live cover of the benthic substrate in the experimental corrals, procedural control corrals and control with no corrals before the restocking of *Diadema antillarum* (November 9, 2021) at Cayo Largo, Fajardo.

% Cover	Experimental	Procedural control	Control
<i>Dictyota</i> spp.	62.67	57.33	65.33
<i>Ramicrosta</i> spp.	22.67	24.89	12.00
Turf	7.11	0.00	0.00
Clean substrate	4.89	0.00	0.00
<i>Palythoa caribaeorum</i>	1.33	0.00	0.00
<i>Caulerpa</i> spp.	0.89	3.56	22.67
Turf with sediment	0.44	1.78	0.00
<i>Halimeda</i> spp.	0.00	1.33	0.00

Palomino

As also observed at Cayo Largo, benthic algae was the dominant substrate on the reef of Palomino (Table 4). However, *Ramicrosta* spp., followed by *Dictyota* spp. was the main contributors to the total abundance of algae. There was slight variation in their contribution between the different corrals, but it was not significant (Index 4). Clean substrate was not present in the procedural controls and the control outside the corrals. Very little amount (mean \pm se, 1.33 \pm 0.67 %) of clean substrate was observed inside the experimental corrals.

Table 4 Mean percent live cover of the benthic substrate in the experimental corrals, procedural control corrals and control with no corrals before the restocking of *Diadema antillarum* (November 9, 2021) at Palomino, Fajardo.

% Cover	Experimental	Procedural control	Control
<i>Ramicrosta</i> spp.	45.78	54.22	81.33
<i>Dictyota</i> spp.	44.44	43.56	18.67
<i>Dictyonella funicularis</i>	3.56	0.00	0.00
<i>Erythropodium caribaeorum</i>	3.11	0.00	0.00
Clean substrate	1.33	0.00	0.00
<i>Cliona</i>	1.33	0.00	0.00
CCA	0.44	0.00	0.00
<i>Halimeda</i> spp.	0.00	0.89	0.00
Turf with sediment	0.00	0.89	0.00
<i>Palythoa caribaeorum</i>	0.00	0.44	0.00

After restocking *Diadema antillarum*

As seen in the models (Index 5), the significance in benthic change through the monitoring period was dependent on whether *D. antillarum* were present inside the corrals at Cayo Largo and Palomino. There was a significant herbivory effect on the change of benthic composition at both sites. These results support the outcomes of previous *D. antillarum* restoration efforts that have taken place all around Puerto Rico (Williams 2016, Williams and Olmeda-Saldaña 2020, Williams 2021). The site specific benthic changes are described below.

Cayo Largo

As seen in the control plots (Fig. 11), the dominant benthic substrate during the first two weeks of the monitoring period was *Dictyota* spp. There was a significant change in benthic cover after the two-week monitoring (Index 1), as the cover changed from *Dictyota* spp. to *Ramicrusta* spp. This was observed in both the procedural control plots and the plots outside a corral. Wave action dictates the distribution of many species of algae, especially fleshy, foliose species like *Dictyota* (Steneck and Dethier 1994). The ocean conditions on the east coast of Puerto Rico are exposed and mainly structured by the north swell and predominant north winds during the winter season. Therefore, the disappearance of *Dictyota* after the two-week monitoring could be caused by the physical abrasion of swells and waves. As observed during the monitoring of the Puerto Rico Coral Monitoring Program (PRCMP), *Dictyota* and turf with sediment mats can overgrow and cover *Ramicrusta* (Williams pers comm). Presumably, *Ramicrusta* was colonizing the benthic substrate underneath *Dictyota* and turf mat. Thus, the abrupt increase of *Ramicrusta* was most likely due to the physical removal of *Dictyota* and turf mats.

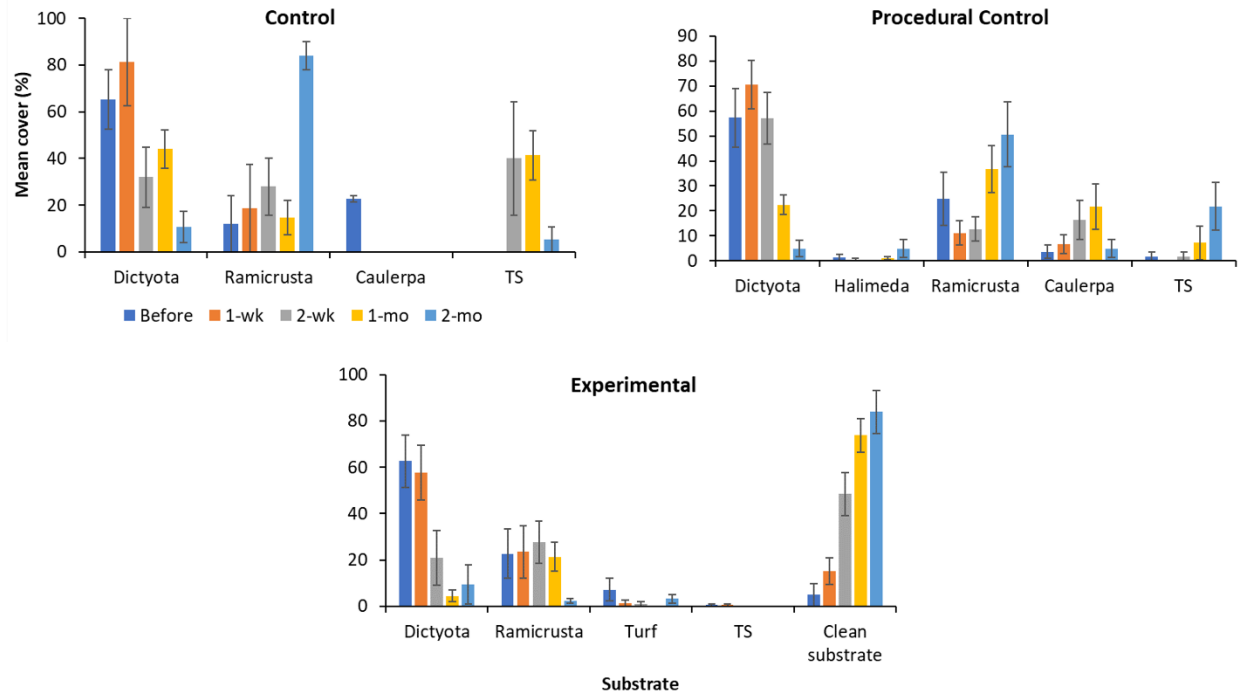


Figure 11 Mean percent cover of the substrate categories inside the experimental corrals with *Diadema antillarum*, the procedural controls which are corrals without *Diadema antillarum*, and control outside the corrals through the monitoring period at Cayo Largo in Fajardo, Puerto Rico.

The restocking of *D. antillarum* has significant impacts on the benthic cover inside the experimental corrals (Index 1). There was a significant difference in benthic assemblage inside the experimental corrals after just two-week after restocking the sea urchins (Index 1). This was due to the reduction of *Dictyota* (SIMPER). After two weeks, *D. antillarum* almost completely removed *Dictyota*, reducing the cover by 93 % and after one month *Ramicrusta* was reduced by 90 % at the end of the monitoring period. As seen in the pictures (Fig. 12, Index 5), the substrate was void of almost any algae by the end of monitoring period. By two months, clean substrate increased by two orders of magnitude, from 4.89 ± 4.89 % to 84 ± 7.15 %

Palomino

In the control plots, the benthic substrate at Palomino was slightly different than at Cayo Largo, as *Ramicrusta* was the dominant category (Fig. 13). However, the cover of *Dictyota* spp.

was still high (43.5 ± 13.9 %) inside the control plots. As seen at Cayo Largo, there was also a significant reduction in *Dictyota* cover after the two-week monitoring inside the procedural controls at Palomino. Palomino is more exposed to oncoming swells and waves than Cayo Largo; therefore, it is not surprising that the same temporal pattern of *Dictyota* was observed at this site.

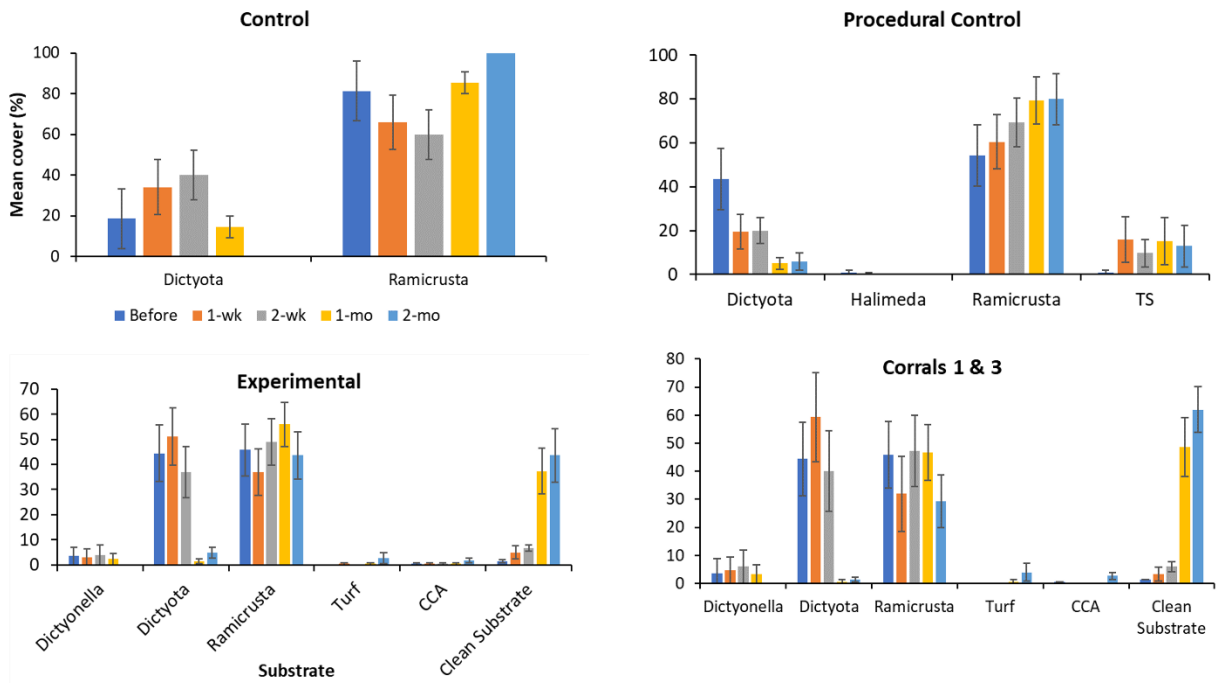


Figure 12 Mean percent cover of the substrate categories inside the experimental corrals with *Diadema antillarum*, the procedural controls which are corrals without *Diadema antillarum*, and control outside the corrals through the monitoring period at Palomino in Fajardo, Puerto Rico.

As seen also at Cayo Largo, the restocking of *D. antillarum* had significant impacts on the benthic cover through time inside the experimental corrals (Index 2). The algal consumption was much slower than at Cayo Largo. As seen in Fig. 13, grazing effects were not noticeable until the one-month monitoring (Index 2). This could be due to the physical environment at this reef. There

was a constant strong bottom surge during the entire monitoring at Palomino. *D. antillarum* were maybe expending most of the energy holding on to the reef, instead of grazing.

All of the *D. antillarum* escaped corral 2 after one-week of the restocking. Therefore, the reduction of benthic algae was not as significant when considering all the experimental plots (see Fig. 13). However, *D. antillarum* significantly reduced *Ramicrusta* by 38 % in corral 1 and 3 after two months (Index 6). *D. antillarum* eliminated an encrusting sponge, *Dictyonella funicularis*, in corral 3 also after two months. Clean substrate increased by two orders of magnitude inside the corrals with *D. antillarum*. In situ observations, confirmed grazing marks on *D. funicularis*. This is the first report of *D. antillarum* consuming this species of sponge, which is a competitor of space (Sammarco et al. 2015).

Conclusions

The natural recovery of *D. antillarum* has not occurred at Palomino or Cayo Largo in Fajardo. Before restocking, the natural populations of *D. antillarum* at Palomino was low (0.03 ind m⁻²), and *D. antillarum* was absent along the southwest part of Cayo Largo. The urchins initially observed at Palomino were large adults with a few individuals present along each spur. The restocking efforts for this project resulted in a one to two order magnitude increase in *D. antillarum* density by the end of the monitoring period. In ecological terms, this is a substantial increase as the natural recovery of populations can take decades (Lotze et al. 2011). However, the result densities (0.73 ind m⁻²) on both reefs are still below the optimal density of 2 to 5 ind m⁻² (Williams per comm, Olmeda-Saldaña et al. 2021). Therefore, multiple restocking events or more than 250 urchins event⁻¹ need to occur at a reef to reach densities of functional significance.

As highlighted in Williams (2021) study, maintaining restocked sea urchins in a specific area of study is a difficult task. Factors affecting the retention of *D. antillarum* are unknown and

need to be further studied. The high retention at Cayo Largo could be due to the high structural relief of the dead *O. annularis* colonies and the low abundance of damselfish. As for Palomino, the structural relief was limited to the cracks and crevices in the reef structure, and individual overhangs of the coral colonies, like *O. faveolata*. Sea urchins were much more hidden at Palomino than at Cayo Largo. This could be due to the higher abundance of damselfish and triggerfish at this site. We believe placing the sea urchins in groups of 30-40 individuals helped their survival during the first couple weeks on the reef. They were in these groups until about a month, and then they dispersed to shelter in cracks or individual coral colonies.

The restocking of *D. antillarum* contributed to positive changes in benthic cover, as these sea urchins significantly reduced the algal cover on both coral reefs in Fajardo. The effects of their herbivory can be observed as early as one week after restocking. Before the restocking of sea urchins, nuisance algae (fleshy macroalgae and peyssonnelids) made up 86 % and 90 % of the benthic cover at Cayo Largo and Palomino, respectively. *D. antillarum* reduced the cover of this nuisance algae by as much as 44 % in one week, and 70 % after one month. This study also reconfirms that *D. antillarum* consumes and significantly reduces *Ramicrusta*. In the experimental plots at Cayo Largo, *Ramicrusta* was almost completely removed from the substrate. The implications of these results are substantial because common day coral reefs are plagued with flesh macroalgae, which hinders any real recovery of the coral populations and other essential benthic invertebrates.

Ramicrusta is detrimental to the health of the reef because it occupies space and quickly overgrows, and smothers live coral tissue. It might also act as a deterrent for coral settlement (Williams pers observ). At Cayo Largo, clean substrate, void of any algae, increased from 5 % to 84 % mean cover in just two months with *D. antillarum*. At Palomino the grazing effects were

slower but still significant, as clean substrate increased from 1 % to 44 % mean cover. Stockton and Edmunds (2021) found coral recruitment to be limited to areas grazed by *D. antillarum* and free of encrusting peyssonnelids. Therefore, coral recruitment may increase at the restoration sites indicating that it should be monitored in the long-term. This study, along with previous *D. antillarum* restoration efforts, prove that this sea urchin is effective in reducing the algal cover on coral reefs, especially *Ramicrusta* spp. and other peyssonnelids. The restocking of *D. antillarum*, and possibly other species of sea urchins (*Echinometra viridis* and *Tripneustes ventricosus*), should be a focus in other restoration programs.

Surveys at Cayo Diablo and Los Lobos

In 2018, *D. antillarum* were restocked to two different Fajardo reefs, Cayo Diablo and Los Lobos. That was the first time that *D. antillarum* were transported from La Parguera to Ceiba. A deliverable for this project was to return to the restoration sites and assess the benthic substrate inside the old corrals and assess the *D. antillarum* populations. Four out of the six corrals were found at Cayo Diablo, while all six corrals were located at Los Lobos. Almost all the nails were missing, making it difficult locating the plots. The *D. antillarum* abundance in the restoration area at Cayo Diablo was low, as only three sea urchins were observed closed to the old corrals. On the other hand, 21 *D. antillarum* were observed close to the plots at Los Lobos. The low number of sea urchins observed during the survey could be due to them migrating to other parts of the reef or mortality, as triggerfish are common at both sites. The low retention of individuals at both sites is contradictory to what was observed at another *D. antillarum* restoration site, Media Luna in La Parguera. At Media Luna, *D. antillarum* densities have continually increased through time from 0 ind m⁻² in 2015 to 13.2 ind 30m⁻² since the restocking in 2016 (Williams 2021).

Given the lack of *D. antillarum* inside the restoration areas, the benthic cover inside the plots at Cayo Diablo and Los Lobos was comprised mostly of *Ramicrusta* and fleshy macroalgae, such as *Dictyota* spp.

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Indices

Index 1 Results from the three-way Permutational Analysis of Variance (PERMANOVA) examining the change in benthic substrate between corral types (CT) and time of monitoring (TI) at Cayo Largo in Fajardo, Puerto Rico. Diadema antillarum (DA) abundance inside the corrals was the covariable. Below are pairwise tests examining the benthic composition inside the experimental and control (including outside) corrals between the time of monitoring.

Source	df	SS	MS	Pseudo-F	p value	
DA	1	50546	50546	5.0277	0.0983	
CT	1	5409.7	5409.7	5.2345	0.011	
TI	4	36077	9019.3	1.4179	0.294	
CTXTI	4	19564	4891	4.7327	0.001	
Experimental corrals			Control (procedural and outside)			
Time	t	p value	Groups	t	p value	
1, 2	1.19	0.241	1, 2	0.95	0.414	
1, 3	2.86	0.007	1, 3	0.78	0.636	
1, 4	4.88	0.001	1, 4	1.88	0.013	
1, 5	4.96	0.001	1, 5	3.30	0.001	
2, 3	1.85	0.064	2, 3	1.51	0.123	
2, 4	3.60	0.002	2, 4	3.10	0.001	
2, 5	3.73	0.001	2, 5	4.78	0.001	
3, 4	1.20	0.248	3, 4	1.22	0.263	
3, 5	1.79	0.062	3, 5	3.14	0.001	
4, 5	1.56	0.109	4, 5	2.04	0.021	

Index 2 Results from the three-way Permutational Analysis of Variance (PERMANOVA) examining the change in benthic substrate between corral types (CT) and time of monitoring (TI) at Cayo Largo in Fajardo, Puerto Rico. Diadema antillarum (DA) abundance inside the corrals was the covariable. Below are pairwise tests examining the benthic composition inside the experimental and control (including outside) corrals between the time of monitoring.

Source	df	SS	MS	Pseudo-F	p value	
DA	1	8942.8	8942.8	1.427	0.3571	
CT	1	9774.6	9774.6	11.194	0.001	
TI	4	18375	4593.8	2.4396	0.09	
CTXTI	4	6765.8	1691.4	1.9371	0.05	
Experimental corrals				Control (procedural and outside)		
Time	t	p value		Groups	t	p value
1, 2	0.30	0.94		1, 2	0.86	0.472
1, 3	1.01	0.385		1, 3	0.73	0.499
1, 4	3.62	0.001		1, 4	1.90	0.04
1, 5	3.01	0.002		1, 5	2.10	0.03
2, 3	0.90	0.439		2, 3	0.54	0.675
2, 4	3.44	0.001		2, 4	1.11	0.294
2, 5	2.74	0.005		2, 5	1.51	0.131
3, 4	2.76	0.002		3, 4	1.35	0.188
3, 5	2.26	0.01		3, 5	1.69	0.102
4, 5	0.85	0.467				

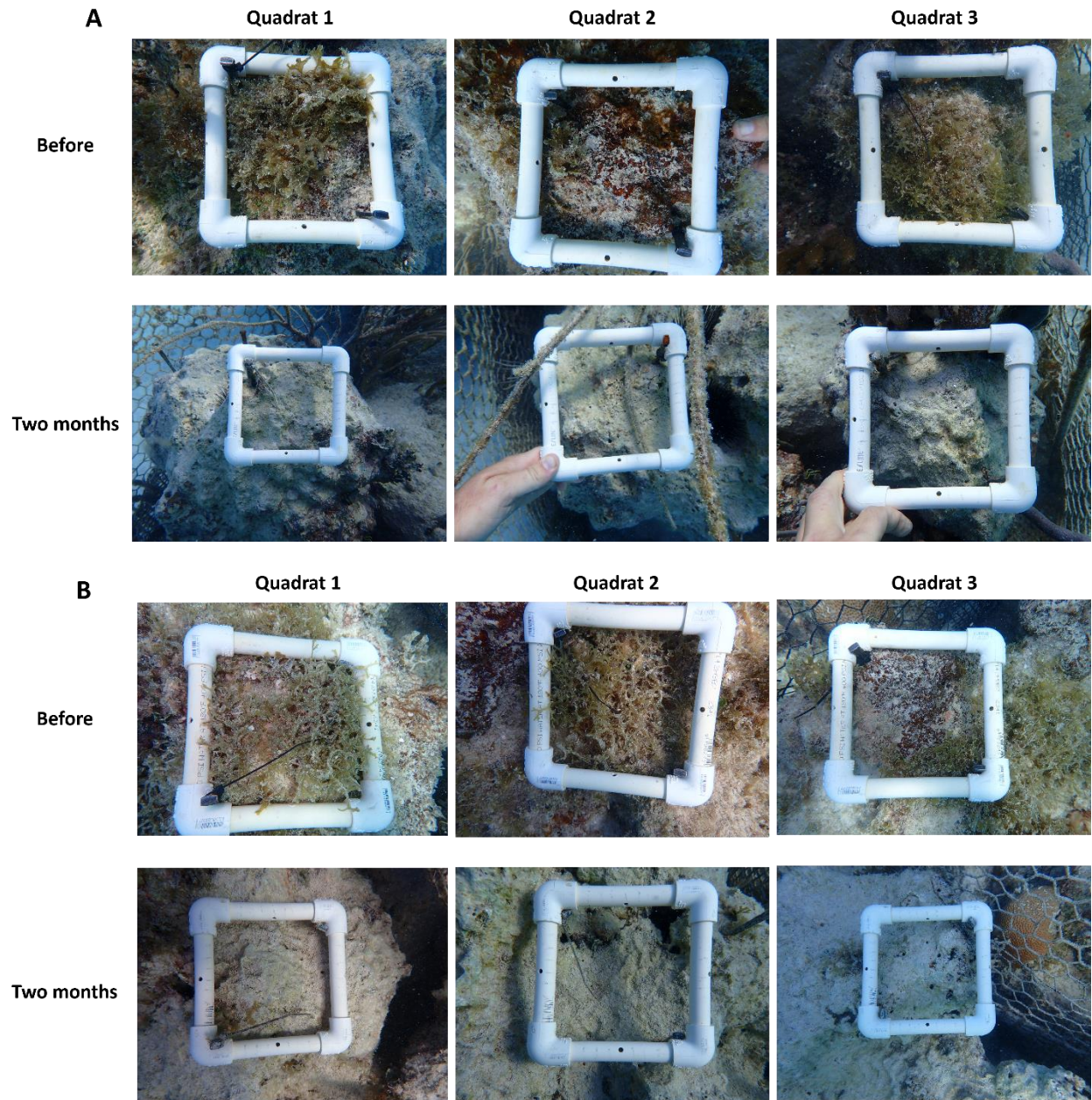
Index 3 The results of the pairwise test to examine the benthic composition between the control and experimental treatments before restocking the Diadema antillarum at Cayo Largo in Fajardo, Puerto Rico.

Groups	t	p value
Control, Experiment	0.87	0.513

Index 4 The results of the pairwise test to examine the benthic composition between the control and experimental treatments before restocking the Diadema antillarum at Palomino in Fajardo, Puerto Rico.

Groups	t	p value
Control, Experiment	0.87	0.426

Index 5 Photographs of the quadrats before Diadema antillarum was restocked and two months after the restocking in corral 1 (A), corral 2 (B), corral 3 (C), and (D) top of the corrals (1, 2, 3) before the restocking of Diadema antillarum and two months after the restocking at Cayo Largo in Fajardo, Puerto Rico.



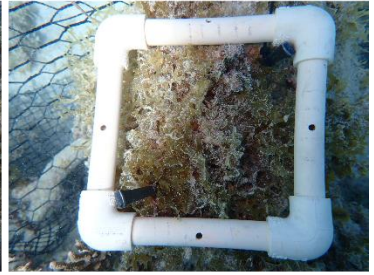
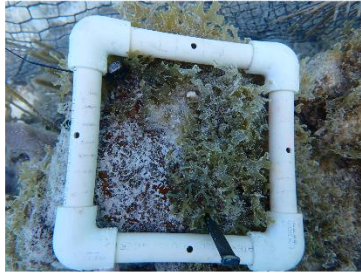
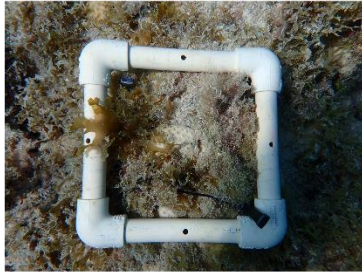
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Quadrat 1

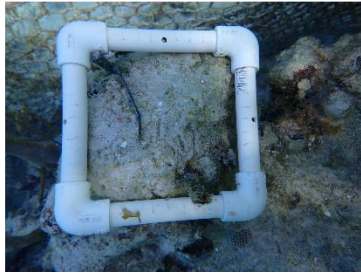
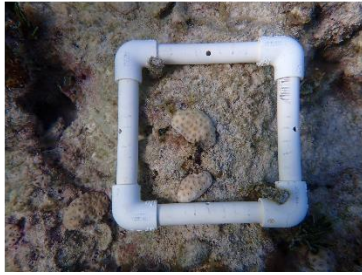
Quadrat 2

Quadrat 3

Before



Two months



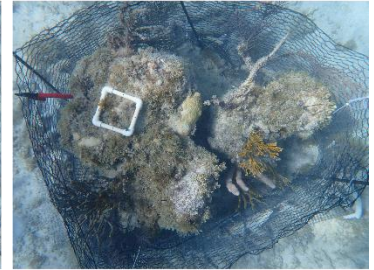
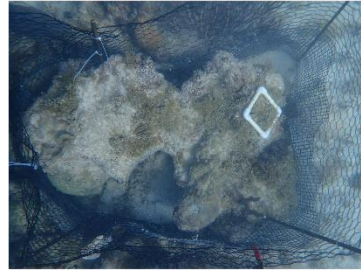
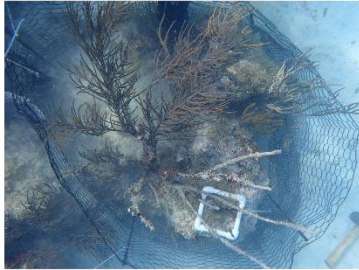
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Corral 1

Corral 2

Corral 3

Before



Two months



Index 6 Photographs of the quadrats before Diadema antillarum was restocked and two months after the restocking in corral 1 (A), corral 2 (B), corral 3 (C), and (D) top of the corrals (1, 2, 3) before the restocking of Diadema antillarum and two months after the restocking at Palomino in Fajardo, Puerto Rico.

