# **Re-stocking of the keystone herbivore species,** *Diadema antillarum***, to reduce algal overgrowth (Phase III)**



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## <span id="page-1-0"></span>Executive Summary

Prior to the restocking event, adult *Diadema antillarum* were absent at both Cayo Largo North (hereafter CLN) and Palomino North (PN) sites. Several factors could have contributed to the low natural abundance of these sea urchins, including exposure to wave energy, low rates of natural settlement or recruitment, and the presence of triggerfish, which are known predators of sea urchins.

Following the restocking event, the retention of *D. antillarum* was generally low within the designated plots and surrounding areas. Urchin abundance varied considerably between monitoring periods both inside and outside the plots. This variability might be attributed to the complex reef structures in the deeper zones, which provided refuge for the urchins, making it difficult for divers to detect and record them. Notably, most restocked urchins moved to deeper areas (>5m) from the initial release depth of approximately 2m. This migration could be a response to suboptimal sea conditions, including rough waters caused by the passing of Tropical Storm Bret.

Before restocking, both sites were characterized by high coverage of the encrusting alga *Ramicrusta*, with a mean cover of 73.1% at CLN and 73.7% at PN. Significant changes in benthic composition were observed at both sites over time, mainly due to reductions in *Ramicrusta* cover. At CLN, *Ramicrusta* cover decreased by 36.5% within two weeks, and at PN, decreased by 40.1% over two months. The reduction of *Ramicrusta* allowed for an increase in turf algae at both sites, with a slight increase in *Dictyota* spp. cover at PN over the sampling period.

In 2021, follow-up assessments were conducted at both CLN and PN using photographic surveys and 20m² benthic transects to measure sea urchin densities. At PN, only two *D. antillarum* were observed near the corral areas, resulting in an overall low mean density of 0.2 ind m<sup>-2</sup>.

Conversely, CLN demonstrated exceptionally high retention, with a mean density of 2 ind m<sup>-2</sup>, the highest recorded since 2015. The high density of *D. antillarum* at Cayo Largo led to a benthic substrate dominated by clean pavement and crustose coralline algae (CCA), indicative of effective grazing pressure by the sea urchins.

The results indicate that *D. antillarum* retention and subsequent ecological impacts vary significantly between sites, influenced by factors such as reef structure, wave exposure, and predator presence. The high retention at Cayo Largo (2021) suggests it is an optimal site for future restocking and coral outplanting efforts due to the effective reduction of nuisance algae and maintenance of cleaner substrates. To enhance restocking success at other locations, it is recommended to consider site-specific conditions, conduct night-time surveys for more accurate urchin counts, and ensure suitable shelter and protection from predators to support *D. antillarum* populations.

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## <span id="page-7-0"></span>Introduction

Over the past four decades, coral reefs in the Caribbean have undergone dramatic changes (Hughes 1994; Wilkinson 2008; Jackson et al. 2014). The abundance of reef-associated organisms, particularly corals, has significantly declined due to a combination of 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 faced additional threats from Stony Coral Tissue Loss Disease (SCTLD) and the proliferation of *Ramicrusta* spp. (hereafter *Ramicrusta*), a harmful encrusting peyssonnelid. *Ramicrusta* has dominated coral cover on the east coast and is slowly spreading to the south and west coasts of Puerto Rico (Williams and García-Sais 2020). This alga threatens slow-growing sessile benthic organisms, such as corals, by overgrowing live tissue (Eckrich and Engel 2013; Ruiz 2015). Moreover, *Ramicrusta* 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* (Williams 2022). However, the keystone herbivore *Diadema antillarum* consumes and can significantly reduce *Ramicrusta* abundance (Williams 2022).

Before the mass mortality event in 1983, *D. antillarum* was common and abundant (13-18 individuals per square meter) on both shallow and deep (>10m) coral reefs in Puerto Rico (Bauer 1980; Vicente and Goenaga 1984). *D. antillarum* played a crucial 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 coral reefs. However, *D. antillarum* populations were drastically reduced by 95-100% in many Caribbean locations (Lessios 1995), which coincided with an increase in fleshy macroalgal cover by 100% to 250% (Phinney et al. 2001). The long-term absence of *D. antillarum* on many reefs has not only influenced benthic algal productivity but has also impacted other reef processes, such as coral recruitment (Hughes et al. 1987).

Currently, the recovery of *D. antillarum* has been slow or non-existent in many areas of the Caribbean (Lessios 2016). In Puerto Rico, there has been a modest recovery (Mercado-Molina et al. 2014; Tuohy et al. 2020); however, densities remain far below pre-mass mortality levels (Lessios 2016) in La Parguera. Tuohy et al. (2020) observed no significant increase in D. antillarum populations from 2001 to 2013. Furthermore, these populations were dominated by medium to large (5-9 cm test diameter) individuals and were primarily found in shallower ( $\leq 5$ m), more structurally complex reefs. Larval and post-recruitment mortality processes may be the main factors limiting adult population sizes of *D. antillarum* (Karlson and Levitan 1990; Williams et al. 2011). In Puerto Rico, it has been shown that upstream sources of "settlement-ready" larvae for *D. antillarum* are available (Williams et al. 2010), suggesting that larval supply and survival are not the main factors inhibiting recovery. Instead, recruitment-limited processes, such as postsettler and 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 coral reefs is a key factor in this lack of recovery, leading to a shift from coral-dominated ecosystems to those dominated by fleshy macroalgae and/or peyssonnelids (Hughes 1994; Shulman and Robertson 1996; McClanahan and Muthiga 1998; Rogers and Miller 2006). Reefs characterized by persistent algal dominance typically exhibit a loss of resilience (Hughes et al. 2007), as macroalgal assemblages limit coral settlement, increase sediment deposition, and alter chemical properties near the benthos (Birrell et al. 2008). Algae not only pose a threat to slow-growing

sessile benthic organisms but also have the potential to reduce the available substrate for coral settlement. Restoring herbivore populations through conservation efforts, such as protection and restocking, is a potential strategy to mitigate 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*, involving 343 young adults (3-4 cm test diameter), took place at the back reef 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 back reef in La Parguera. Significant changes in benthic structure were observed within the first week after restocking in all restoration projects, including notable reductions in fleshy macroalgae (*Dictyota* spp.) and thick turf algal/sediment mats (TAS), both of which are unsuitable substrates for coral settlement. Restocked *D. antillarum* also significantly reduced the cover of encrusting red algae, *Ramicrusta*. By the end of the studies in 2018 and 2019, the abundance of fleshy macroalgae decreased by a mean of 77% (up to 100%), while *Ramicrusta* and TAS decreased by 53% (up to 71%) and 56% (up to 100%), respectively. The presence of clean substrate ("pavement"), crustose coralline algae (CCA), and filamentous turf algae increased by one to two orders of magnitude (Williams 2022).

Similar results were reported for the D. antillarum restocking in 2020-2021 at Cayo Largo south and Palomino. 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  $4.89 \pm 4.89$  % to  $84 \pm 7.15$  % (Williams et al. 2021). Through restocking, *D. antillarum* densities (mean ± se) increased from one order of magnitude at Palomino  $(0.03 \pm 0.02 \text{ ind m}^{-2} \text{ to } 0.73 \pm 0.13 \text{ ind m}^{-2})$  to two orders of magnitude at Cayo Largo (0 ind m<sup>-2</sup>) to  $0.73 \pm 0.46$  ind m<sup>-2</sup>).

The goals of this project was to continue restocking *D. antillarum*, by increasing their population at two coral reefs in Fajardo, specifically at Cayo Largo North and North Palomino. Additionally, this project aimed to document the herbivory effects of restocked *D. antillarum* on the abundance of nuisance algae, such as fleshy macroalgae (e.g., *Dictyota* spp.) and *Ramicrusta*. Lastly, I revisited 2021 restoration sites, Cayo Largo and Palomino (Sand Slide) to assess the *D. antillarum* retention.

## <span id="page-10-1"></span><span id="page-10-0"></span>**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. The receiving tanks were modified 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



<span id="page-10-2"></span>*Figure 1 Figure 1 Diadema antillarum settler (~1 mm test diameter).*

<span id="page-10-3"></span>

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

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 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 ℃ , 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.*

Settlers were transferred to wet tables or raceways 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

<span id="page-11-0"></span>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*, *Stypopodium*, *Ulva sp.* and *Dictyota* spp., were collected in the field once a week and kept in a holding tank.

In November 2022, 793 lab-reared *D. antillarum* and 190 *Tripneustes ventricosus* were transferred from La Parguera to our other sister land-based nursery at Ceiba called the Centro de Investigación y Restauración de Organismos Marinos (CIROM, Fig. 4). We transferred another 656 *D. antillarum* and 81 *T. ventricosus* in March 2023 to the Ceiba nursery.



*Figure 4 CIROM-Ceiba staff receiving the sea urchins from CIROM-La Parguera. Checking water quality in each bucket before acclimation.*

<span id="page-12-0"></span>Therefore, the nursery was housing a total of 1,449 *D. antillarum* and 271 *T. ventricosus* (1,720 total urchins). The Ceiba land-based nursery contains 12 rearing (grow-out) tanks with sumps in close recirculating systems (Fig. 5). Fresh seawater is cleaned through a mechanical and UV sterilizer. One hundred percent water exchanges are conducted on a daily basis for each tank. Tanks are cleaned on a regular basis and sea urchins are fed with *Ulva* a couple of times a week.



*Figure 5 Tanks at the land-based nursery in Ceiba, Puerto Rico.*

#### <span id="page-13-1"></span><span id="page-13-0"></span>Restoration sites

Cayo Largo North reef (CLN: 18°18'.857 N, 065°34'.772 W) is a long, partially submerged fringing reef located southwest of Palomino reef (Fig. 6). Corrals were installed on the leeward side of the reef on the northern part at 3-4 meters of depth. The reef slope was gentle, with *Acropora palmata* colonies present and abundant in the shallows and large, dead *Orbicella annularis* colonies at the base of the reef. Sand and *Thalassia testudinum* plains bordered the deeper parts of this reef. *D. antillarum* was absent along the three, 20 m<sup>2</sup> belt transects conducted in the area of restocking. Also, no triggerfishes were observed.

Palomino North reef (PN: 18°21'.308 N, 065°34'.421 W) is composed of fringing patch reefs separated by sand channels and is within the Arrecifes de la Cordillera in Fajardo. This restocking site is highly exposed to wave action and the north swells and a site where Sea Ventures and CariCOOS is measuring wave attenuation. Sea Ventures outplanted *A. palmata* and coral microfragments to measure the impacts these corals will have on wave reduction. Plots were installed on the top of the reef patch, in depths of 3m and in areas of coral outplants. Given the exposure of this site, the substrate is relatively flat pavement. Rugosity increased on the slopes of the patches. *D. antillarum* were not present at the restoration sites. However, one ocean triggerfish (*Canthidermis sufflamen*) was observed about 500-600 meters from plots.



*Figure 6 Map of the restocking sites, Cayo Largo North and North Palomino in Fajardo, Puerto Rico.*

#### <span id="page-14-1"></span><span id="page-14-0"></span>Plot installation

On June 21, 2023, five, 1m-long 3/8" rebars were randomly installed at CLN and PN. Three 25 cm x 25 cm fixed quadrats were installed close to each rebar (Fig. 7). Nails marked the position of fixed quadrats, allowing for the estimation of change. To monitor the change in benthic substrate cover before and after *D. antillarum* restoration, Photographs were taken before restocking (June 21), one week (June 28), two weeks (July 5), one month (July 26) and two months (August 23) after restocking.



*Figure 7 Two divers installing the permanent the nails to mark the permanent quadrats at Cayo Largo North in Fajardo, Puerto Rico.*

<span id="page-15-1"></span>*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.

#### <span id="page-15-0"></span>Data analysis

A three-way distance Permutational Multivariate Analyses of Variance (PERMANOVA) tests (Anderson 2001) were performed to assess the differences in benthic composition between site (CLN, PN), plots (5 in total) and time (5 in total). 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. 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.

### <span id="page-16-0"></span>Results

#### <span id="page-16-1"></span>Sea urchin retention

Given the possibility for Tropical Storm Bret to make landfall, the team decided to release *D. antillarum* on June 21, 2023. Early in the morning, 628 lab-reared *D. antillarum* were removed from tanks at ISER's land-based nursery, CIROM, in Ceiba to multiple round bins and placed in Pure Adventure boat (Fig. 8). The benthic composition was recorded before and after the sea urchin restocking at each site. Sixty *D. antillarum* were released close to each plot (5 in total) at PN and CLN.

<span id="page-16-2"></span>

*Figure 8 Diadema antillarum were transferred from land-based nursery at the Centro de Investigación y Restauración de Organismos Marinos (CIROM) on June 21, 2023.* 

Before the restocking event, adult *D. antillarum* were absent at both sites. The low natural abundance of *D. antillarum* at both sites could be due to level of exposure to wave energy, low natural settlement/recruitment and the abundance of triggerfish in the area. As seen in Figure 9, retention of urchins after restocking was low inside the plots and the surrounding area. In addition, urchin abundance varied greatly between monitoring periods inside and outside the plots. The variation between monitoring periods could be due to the reef structure, as both reefs were highly complex in the deeper zones. This complex structure provided refuge for the urchins to hide. Therefore, making it difficult for the divers to observe and record them. I did notice that the majority of restocked *D. antillarum* observed during the monitoring moved to deeper areas ( $>5$ m). The area where the urchins were released was shallow ( $\sim$ 2m). In addition, the day of restocking the sea conditions were not ideal due to the passing of Tropical Storm Bret. It is very plausible, the majority of sea urchins took refuge in deeper areas due to the well and rough weather conditions, as no dead urchins were reported during the monitoring. The abundance of sea urchins will most likely increase through time as the urchins get bigger and are easier to observe.



<span id="page-17-0"></span>*Figure 9 The abundance of lab-reared Diadema antillarum restocked at Palomino North and Cayo Largo North in Fajardo during the different monitoring periods. Sea urchins outside the plots are included in the totals.*

#### <span id="page-18-0"></span>Benthic composition

At both sites, the benthic composition before the sea urchin restocking, was characterized by high abundance of *Ramicrusta* (Mean  $\pm$  SE), 73.1  $\pm$  4.7% at CLN and 73.7  $\pm$  2.5% at PN, followed by turf alga (Fig. 10).



<span id="page-18-1"></span>*Figure 10 Mean cover (%) of the benthic substrate at different sampling times at Cayo Largo North and Palomino North in Fajardo, Puerto Rico.* 

Significant changes in benthic composition were observed at both sites through time, and as seen in pair-wise analyses, these changes were observed two weeks after *D. antillarum* were released at PN, and one month at CLN (Table 1). The reduction in *Ramicrusta* cover drove these significant differences. At Cayo Largo, the cover of *Ramicrusta* decreased by 36.5% in two week, while at Palomino it decreased by 40.1% in two months (Fig. 10). Turf alga cover increased at both sites with the reduction of *Ramicrusta*. At Palomino, *Dictyota* spp. cover also slightly increased through sampling time.

<span id="page-19-1"></span>*Table 1 Results from the three-way Permutational Analysis of Variance (PERMANOVA) examining the change in benthic substrate between sites (Si), plots (Pl) and time (Ti) before and after restocking Diadema antillarum in Fajardo, Puerto Rico. Table below highlights the results from a pairwise test examining the benthic composition between sampling time at Cayo Largo North and Palomino North in Fajardo, Puerto Rico. 1=before, 2= one week after, 3= two weeks after, 4= one month after, and 5= two months after restocking.* 

<b>Source</b>	df	<b>SS</b>	MS	<b>Pseudo-F</b>	$P(\text{perm})$
Si		5513	5513	2.1938	0.165
P1	4	6154.7	1538.7	5.1672	0.001
Ti	4	6280.9	1570.2	6.4159	0.001
<b>SixPl</b>	4	9818.1	2454.5	8.2428	0.001
<b>SixTi</b>	4	1657.5	414.37	1.6893	0.137
PlxTi	16	3799.9	237.49	0.79755	0.827
<b>SixPlxTi</b>	15	3619.4	241.29	0.81031	0.795



#### <span id="page-19-0"></span>2021 *D. antillarum* restocking sites

I revisited the *D. antillarum* restocking sites in 2021, Palomino (Sand Slide) and Cayo Largo and took photographs of the permanent quadrats. Three  $20m^2$  benthic transects were also conducted to measure sea urchin densities. Only two *D. antillarum* were observed in areas where the corrals were located at Palomino, close to corral 1 and 2. The overall mean density of *D. antillarum* in the restoration area was  $0.2 \pm 0.01$  ind m<sup>-2</sup>. On the other hand, the retention of *D*. *antillarum* at Cayo Largo was probably the highest ever recorded for a site since 2015. The mean density of *D. antillarum* at this site was  $2 \pm 0.01$  ind m<sup>-2</sup>. Given the high abundances of *D*.

*antillarum*, the benthic substrate was dominated by clean pavement and crustose coralline algae (CCA) (Fig. 11).



*Figure 11 Photographs taken in 2024 at Cayo Largo in Fajardo, a site where Diadema antillarum were released in 2021.*

## <span id="page-20-1"></span><span id="page-20-0"></span>Discussion and conclusions

Overall, the retention of *D. antillarum* at both study sites was low, with their abundances fluctuating significantly throughout the sampling period. This variability could be attributed to several ecological and behavioral factors. Surveys were conducted during the mornings when *D. antillarum* typically seek shelter in holes and crevices within the reef structure, making them less visible and potentially leading to underestimates of their actual population sizes. Conducting surveys at night, when these sea urchins are more active and venture out of hiding to graze, would likely provide a more accurate representation of their retention and distribution across the study sites.

The challenges associated with tracking *D. antillarum* retention in specific study areas have been highlighted in previous research, including the study by Williams (2021). Maintaining restocked sea urchins within a designated area is inherently difficult due to their natural behavior and the complex dynamics of reef ecosystems. *D. antillarum* are known to migrate across the reef in search of food, shelter, and optimal environmental conditions, which can lead to their dispersion beyond the immediate vicinity of the restocking sites.

Several factors that influence the retention of *D. antillarum* warrant further investigation to improve the success of restocking efforts. One critical factor is the abundance of damselfish, which are territorial and often aggressive towards other reef organisms, including sea urchins. High densities of damselfish may limit the availability of suitable grazing areas for *D. antillarum*, thereby affecting their retention and survival rates. Additionally, damselfish are known to cultivate algal gardens, which could reduce the amount of available substrate that *D. antillarum* need for feeding.

The amount and quality of available shelter are also crucial for *D. antillarum* retention. These sea urchins require adequate hiding places to protect themselves from predators such as fish and invertebrates. Palomino North reef did not provide much shelter, which may force *D. antillarum* to migrate in search of safer habitats, resulting in lower retention rates in the study areas. Structural complexity of the reef, including the presence of crevices, overhangs, and coral rubble, plays a vital role in providing refuge and maintaining healthy *D. antillarum* populations.

High retention rates of *D. antillarum* are evident at the Cayo Largo 2021 site, where the majority of the sea urchins are still present three years after the initial restocking event. This site offers several favorable conditions that are likely to contribute to the successful retention and survival of *D. antillarum*. One of the key factors is the high structural relief of the reef, which primarily consists of dead *O. annularis* colonies. These structures provide ample hiding spaces and crevices that *D. antillarum* utilize for protection against predators and environmental stressors, enhancing their chances of survival. The complexity of the reef structure not only offers shelter but also stabilizes the sea urchins' preferred habitats, making it less likely for them to migrate to other areas.

In addition to the high structural relief, the Cayo Largo site is characterized by a low abundance of damselfish, particularly the threespot damselfish (*Stegastes planifrons*), which are known to be rare in this area. Damselfish can be highly territorial and aggressive, often competing with other reef organisms for space and resources. They cultivate algal gardens, which can lead to increased competition for grazing areas and negatively impact *D. antillarum* by limiting their access to essential food resources. The low damselfish population at Cayo Largo minimizes these competitive interactions, allowing *D. antillarum* to graze more effectively and maintain higher densities.

The presence of a high density of *D. antillarum* (approximately 2 ind.  $m<sup>-2</sup>$ ) at the Cayo Largo site has had significant ecological implications for the reef. Their grazing activity has led to the almost complete absence of *Ramicrusta* spp., a harmful encrusting alga, and fleshy macroalgae, which are both known to outcompete corals for space and resources. By effectively controlling these nuisance algae, *D. antillarum* create a more favorable environment for coral recruitment and growth. The reduction of *Ramicrusta* and fleshy macroalgae helps to keep the reef substrate cleaner, promoting the settlement of coral larvae and the recovery of coral populations.

Given these conditions, the Cayo Largo site presents an excellent opportunity for coral outplanting initiatives. The high retention of *D. antillarum* ensures ongoing grazing pressure that helps maintain low levels of competitive algae, thereby providing suitable conditions for coral larvae to settle and thrive. Moreover, the structural complexity of the reef, combined with the reduced competition from damselfish, offers an ideal environment for coral transplants to establish and grow. Coral outplanting at this site could accelerate reef recovery, enhance biodiversity, and contribute to the restoration of reef structure and function.

In conclusion, the Cayo Largo 2021 site exemplifies a successful model for *D. antillarum* restocking and demonstrates the potential benefits of such efforts in restoring coral reef health. By maintaining high urchin densities and minimizing algal overgrowth, this site serves as a valuable reference for similar projects elsewhere. Future research and conservation efforts should focus on replicating these favorable conditions to promote coral reef resilience and recovery across the Caribbean.

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# <span id="page-31-0"></span>Indices

<span id="page-31-1"></span>*Index 1 Photographs of the quadrats before Diadema antillarum was restocked (left) and two months after the restocking (right) in each plot (5 in total) at Cayo Largo North in Fajardo, Puerto Rico.*











Plot 4







<span id="page-33-0"></span>*Index 2 Photographs of the quadrats before Diadema antillarum was restocked (left) and two months after the restocking (right) in each plot (5 in total) at Palomino North in Fajardo, Puerto Rico.*



Plot 2





#### Plot 4





<span id="page-36-0"></span>*Index 3 Photographs taken top down of corral 3 before Diadema antillarum was released in 2021 (left) and again in 2024 in Cayo Largo in Fajardo, Puerto Rico*





