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The reduction of harmful algae on Caribbean coral reefs through the reintroduction of a keystone herbivore, the long spined sea urchin, *Diadema antillarum*

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Running head: Grazing effectiveness by *Diadema antillarum*

Abstract

Herbivores play an essential role in the health and recovery of a coral reef ecosystem. The lack of recovery of the keystone herbivore, *Diadema antillarum*, has had long-lasting effects, evidenced with many reefs persisting in algal dominance. This study restocked 756 lab-reared *D. antillarum* to four coral reefs on the east and south coast of Puerto Rico. Sea urchins were placed in experimental plots ("corrals") for two months, and the change in benthic composition was measured. Significant changes in the benthic structure were observed during the first week after the restocking. 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, *Ramicrusta* spp. By the end of the study, the abundance of fleshy macroalgae decreased by a mean of 77% (max of 100%) and *Ramicrusta* 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. The restoration of native sea urchins is a non-invasive and useful approach to aid in the mitigation of

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algae, especially potentially dangerous alga like *Ramicrusta*. The results of this study highlight the importance of herbivores in improving the conditions on coral reefs.

Keywords: *Diadema antillarum*, sea urchin, herbivore, restoration, coral reef, Caribbean

Implications for Practice:

- Coral restoration has primarily focused a restocking scleractinian corals. The reef substrate housing the coral fragments must be manually cleaned by divers to remove algae before and after outplanting. As seen in this study, restocked *Diadema antillarum* effectively reduces benthic algae. *D. antillarum* can be restocked simultaneously with corals to aid in restoration success.
- *D. antillarum* is the only known consumer of harmful peyssonnelids, like *Ramicrusta*. *D. antillarum* can be restocked to coral reefs experiencing a high abundance of *Ramicrusta*.
- Post-larval capture and culture is an alternative technique to produce individuals in the laboratory for restoration purposes.
- Further studies are needed to understand the habitat preferences of *D. antillarum* to enhance the retention of restocked individuals.

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 & Sheppard 1993; Littler et al. 1993; Hughes 1994). 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

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D. antillarum occurred throughout the Caribbean basin and was one of the most extensive and severe die-off ever recorded for a marine invertebrate (Lessios 1995).

Before 1983, *D. antillarum* was common (13-18 ind m^{-2}) on coral reefs in Puerto Rico (Bauer 1980; Vicente & Goenaga 1984). They played an important role in structuring coral reef communities by controlling algal abundance (Carpenter 1981; Odgen et al. 1973; Sammarco 1982), and productivity (Williams 1990). Given their high numbers at the time, *D. antillarum* was one of the principal agents of bioerosion on reefs (Lidz & 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 long absence of *D. antillarum* has not only influence the benthic algal productivity of coral reef communities, but it also has influenced 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. 2015, 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 surveys, and populations were dominated by medium to large (5-9 cm test diameter) individuals that were concentrated at shallower (< 5 m), more complex reefs. Larval mortality and/or post-recruitment mortality processes could be the bottlenecks regulating the recovery of *D. antillarum* to coral reefs (Karlson & Levitan 1990; Williams et al. 2010). In Puerto Rico, the larval supply and survival do not seem to be inhibiting the recovery of these populations (Williams et al. 2010). Consequently,

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recruitment-limited processes, such as post-settler and/or juvenile mortality, maybe inhibiting the recovery of *D. antillarum* in Puerto Rico (Williams et al. 2011).

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; Rogers & 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). Reef degradation in Puerto Rico is occurring at a rapid pace (Ballantine et al. 2008, Weil et al. 2009). Reefs are now characterized by a high abundance of fleshy macroalgae and turf algae that have monopolized substrate previously covered by corals and other sessile reef invertebrates.

There is a new threat to the coral reefs in Puerto Rico, *Ramicrusta* spp. *Ramicrusta* is an encrusting red alga from the Peyssonneliaceae family (Rhodophyta). Currently, there are three species of *Ramicrusta* identified in the Caribbean; *textilis*, *bonairensis*, and *monensis* (Ballantine et al. 2011). *Ramicrusta* forms a thin, crustose layer that spreads over the substrate and can grow over the living tissues of other organisms (Pueschel & Saunders 2009; Eckrich & Engel 2013). Not much is known of the origins of this alga, and the reasons for its increase around the Caribbean is unknown. The overgrowth rate is high for this alga, ranging between 0.06 mm d^{-1} and 0.08 mm d^{-1} (Eckrich & Engel 2013). The spread of *Ramicrusta* has been occurring throughout Puerto Rico (Williams and Garcia-Sais 2020). In Puerto Rico, the abundance of *Ramicrusta* varies between coasts, with the east coast having a high cover, ranging between 45-75% (Williams & Garcia-Sais 2020). The rapid growth of *Ramicrusta* makes this alga not only a threat to slow growing sessile-

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benthic organisms but has the potential to reduce the area of suitable substratum for coral settlement. Coral species listed under the Endangered Species Act (ESA) such as, *Acropora* and *Orbicella*, have been negatively impacted by the expansion of *Ramicrust*a (Ruiz 2015; Williams & García-Sais 2020).

D. antillarum has been witnessed eating *Ramicrusta* in the laboratory (S. M. Williams, 2018, Institute for Socio-Ecological Research, personal observation), and some studies have concluded these sea urchins might be eating these algae in the field (Ruiz 2015; Williams & García-Sais 2020). In this paper, I describe the changes in the benthic assemblages after increasing the densities of *D. antillarum* to four coral reefs in Puerto Rico. Coral reefs targeted for this project were characterized by a high abundance of nuisance algae, such as *Ramicrusta*, fleshy macroalgae (*Dictyota* spp.), and thick turf mats with sediment. Also, this study aimed to confirm that *D. antillarum* consumes *Ramicrusta* in the field.

Methods

The sea urchins restored to the coral reefs for this project were from settlers collected in the field and grown in the laboratory (Williams 2016). The supply of *D. antillarum* settlers was collected at a shelf-edge reef in La Parguera, Puerto Rico. The methodology of settler collection is outlined in Williams et al. (2010, 2011) studies. Settlers were brought back to the laboratory and grown in the wet-lab facility at the Department of Marine Science at the University of Puerto Rico, Mayagüez. The settlers (<1 mm in test diameter) were grown in semi-closed raceways and fed a mix of algae such as *Dictyota* spp. , *Ulva* sp., *Acanthophora* sp. and *Padina* sp. Settlers were reared in the tanks from 10 to 12 months, until they reached young adult stage (3-4 cm in test diameter).

Lab-reared *D. antillarum* were reintroduced to four reefs in Puerto Rico. In August 2018, *D. antillarum* juveniles were transferred from the laboratory to the backreefs of Cayo Diablo

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(N18°21’37.37, W65°31’59.7) and Los Lobos (N18°22’26.58, W65°34’12.9) in Fajardo (Fig. 1) and in August 2019, to the backreefs of El Coral (N17°56’55.1, W67°1’4.73) and at Mario (N17°57’11.23, W67°3’22.82) in La Parguera (Fig. 1). These coral reefs were chosen for herbivore restoration because they were dominated by algae, specifically *Ramicrusta* spp. (hereafter “*Ramicrusta*”), fleshy macroalgae (*Dictyota* spp.) and turf algal/sediment mats (hereafter “TAS”). TAS substrate is created when turf algae grow in thick mats that trap sediment. Also, the natural populations of *D. antillarum* on all reefs were low (<0.01 ind m^{-2}). All reefs were in 5-7 meters of depth and were characterized by mostly dead *O. annularis* colonies surrounded by sandy habitats.

Before restoration, six corrals were installed at each reef and held into place with rebar. Corrals were necessary to measure the changes in benthic composition because the urchins disperse if released freely. Sometimes they move far (>30m) from the area of reintroduction (SM Williams, personal observation). The diameter of each corral was approximately 2.4 m (4.5 m^2 area). Corrals were made of galvanized chicken wire with a 1-inch diameter mesh size. The plastic chicken netting was attached to the bottom of the corral to mold to the reef (Fig. 2). Corrals were placed in the sand around isolated *O. annularis* colonies. Tops were temporarily placed on corrals for the first two weeks after restocking. Tops were removed after two weeks. The reason for the tops was to reduce the number of urchins from escaping. Past restoration activities have shown that sea urchins start displaying homing behavior after two weeks of reintroduction (S.M. Williams personal observation). All corrals and rebars were removed from the reef after two months of monitoring.

On 22nd August 2018, 480 lab-reared *D. antillarum* were transferred to Fajardo. Twenty-five (25) *D. antillarum* were placed in each corral (5.5 ind m^{-2}), and the rest of the sea urchins were released freely on the reef. In 2019, a total of 276 sea urchins were transferred to El Coral

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and Mario on 31st August 2018. Twenty-three (23) sea urchins were placed in each of the corrals (5.1 ind m^{-2}). To monitor benthic change through time, I photographed three fixed, 25 cm x 25 cm quadrats in each corral. There were three fixed and three random placed quadrats were photographed in each corral. Fixed quadrats were placed in areas with high algal cover, especially *Ramicrusta*. Masonry nails nailed into the substrate and 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. One limitation of this study is that there were no procedural controls (corrals without sea urchins) installed. However, past research in Puerto Rico demonstrates that there are no caging ("corral") effects on the abundance of algae in a six-month period, even when the corrals were fully enclosed (Olmeda-Saldaña et al. 2021, Williams and Olmeda-Saldaña 2021). Corrals were placed between five and ten meters apart, and the controls were placed 20 to 30 m away from corrals. The 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 (CPCe). In CPCe, 50 points were placed in a uniform grid for the fixed photo-quadrats, and 50 points were randomly placed for the haphazard photo-quadrats. Photographs were taken before restocking the sea urchins and then one week, two weeks, one month, and two months after the reintroduction. Photographs were not taken at two weeks at Fajardo sites and after one month at Los Lobos because of a camera malfunction.

A three-way Permutational Multivariate Analysis of Variance (PERMANOVA) test (Anderson 2001) for each location (Fajardo and La Parguera). For the three-way PERMANOVAs, the changes in benthic taxonomic structure were examined between sites (Fajardo: Cayo Diablo and Los Lobos, La Parguera: Mario and El Coral), plots (six corrals with *D. antillarum* and control

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without *D. antillarum*), and sampling time. Plots were nested within sites (random). For analyses, random quadrats were considered non-independent through time because of the limited sample area inside each corral (higher chance of sampling the same area through time). Therefore, permanent and random quadrats were pooled for the analyses and the data were square-root transformed to reduce the influence on the dominant taxa (Anderson et al. 2008). Sea urchin abundance inside each corral during each sampling time was used as a covariate in the analyses. 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 and Monte Carlo simulations were included. A three-way PERMANOVA was used to assess the differences in *Ramicrusta* cover between the sites, plots (nested in sites) and sampling time. This analysis was only conducted for Fajardo because *Ramicrusta* was absent in the plots in La Parguera. For the analyses of *Ramicrusta* cover (univariate), the similarity matrix was based on Euclidean distances. Euclidean distance measures for univariate PERMANOVA analyses produce sums-of-squares estimates equivalent to parametric ANOVA (Anderson 2001). All data were square-root transformed and sea urchin abundance was used as a covariate in the analyses. Pair-wise comparison tests were performed to identify the variation in benthic cover inside the control plots through time in each of the models. SIMPER tests were run to identify the contribution of benthic categories to the overall differences between the variables. The multivariate statistical tests were carried out with the PRIMER v.6 with PERMANOVA add-on software (Anderson et al. 2008; Clarke & Gorley 2015).

Results

Retention of restocked individuals

The retention of restocked *D. antillarum* inside corrals decreased through time, independent of location or site. As seen in Figure 3, most of the sea urchins escaped the corrals after one-week of restocking. The mean retention of sea urchins inside the corrals was similar between La Parguera (27%) and in Fajardo (26%), however varied between sites. El Coral showing the greatest densities by month two (43% retention).

Benthic change

Fajardo

Before the reintroduction of *D. antillarum*, the benthic composition between the corrals and controls was similar, both treatments were mainly characterized by the high abundance of *Ramicrusta* at both Cayo Diablo (Fig. 4a) and Los Lobos (Fig. 4b), with means (±SE) ranging from 30 ± 9.9% at Cayo Diablo to 80 ± 4.8% at Los Lobos. Filamentous turf algae (hereafter "turf algae") and *Dictyota* spp. were also abundant outside and inside corrals. Inside the corrals, the cover of other benthic organisms was low, especially that of coral cover, which ranged in cover between 0% to 14.7 ± 7.5% at Cayo Diablo to 0% to 13.7 ± 8.9% at Los Lobos.

In the control plot, benthic composition did not show any significant changes through time at Cayo Diablo and Los Lobos. By the end of the study, the benthic composition was distinctly different inside the experimental plots compared to the controls (Fig. 4a, b). In the experimental plots, the changes in benthic composition were dependent on the number of *D. antillarum* inside the plots (three-way PERMANOVA, Pseudo-F=10.34, P(MC)=0.001). The grazing effects by the sea urchins varied significantly between sites, plots and sampling time (three-way PERMANOVA, P(MC)<0.05).

The benthic composition in the control plots did not significantly differ after one week of the restocking (three-way PERMANOVA, P(MC)=0.17). However, benthic composition did significantly change within one week after the reintroduction of sea urchins in the experimental plots. Benthic substrate was characterized by more turf algae and clean substrate (hereafter

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"pavement") at both sites after one week. *Ramicrusta* was reduced by 44% at Cayo Diablo and 62.6% at Los Lobos (Fig. 5a, b). There was also a lower abundance of fleshy macroalgae, specifically *Dictyota* spp. 14.8% at Cayo Diablo (Fig. 5a). There was a more marked shift in benthic community structure after two months of restocking, and this was independent of sites. The benthic substrates that contributed to the change in composition over time were *Ramicrusta*, pavement, *Dictyota* spp., CCA, and turf algae (SIMPER). The cover of *Ramicrusta* inside the control plots did not significantly differ through the monitoring (three-way PERMANOVA, P(MC)=0.69). For the experimental corrals, the differences in benthic assemblage after one and two months was due to the change in *Ramicrusta* abundance (Fig. 5a, b). *D. antillarum* significantly reduced *Ramicrusta* (three-way PERMANOVA, P(MC)<0.05), which was replaced by pavement and turf algae (Fig. 6). The rate at which *D. antillarum* significantly reduced the cover of *Ramicrusta* through the monitoring period (three-way PERMANOVA, P(MC)<0.05). *Ramicrusta* was reduced on average by 46% at Cayo Diablo and 51% at Los Lobos by the end of the study. Turf algae and pavement increased by 191% and 669% (respectively) at Cayo Diablo and by 114% and 182% at Los Lobos.

La Parguera

Similar to Fajardo, the benthic composition was not significantly different between the controls and experimental plots before the reintroduction of *D. antillarum* (Fig. 7 a, b). Before the restocking, the benthic substrate at El Coral was characterized by a high mean cover (± SE) of *Dictyota* spp. of 36.9 ± 4.2% and turf mats, 23.1 ± 3.8%. Other fleshy macroalgae, such as *Padina* spp., and articulate calcarous algae, *Halimeda* spp., were common. Crustose coralline algae (CCA) and pavement were low at both sites. At Mario, the dominant substrate in the quadrats before the restocking was turf algae with a mean cover of 29.6 ± 4.0%, *Dictyota* spp.

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(16.1 ± 2.9%) and unidentified fleshy macroalgae (14.5 ± 3.1). There was no CCA and pavement present in the corrals before restocking.

The benthic composition in the control plots did not differ through the monitoring (three-way PERMANOVA, P(MC)=0.15). By the end of the study, the benthic composition was distinctly different inside the experimental plots compared to the controls (Fig. 7a, b). In La Parguera sites, the changes in benthic composition of the experimental plots were dependent on the number of *D. antillarum* (three-way PERMANOVA, Pseudo-F=4.82, P(MC)=0.025). The grazing effects by the sea urchins varied significantly between sites, plots and sampling time (three-way PERMANVOA, P(MC)<0.05).

At both El Coral and Mario, the grazing effects of *D. antillarum* significantly changed the benthic composition inside the corrals. These changes were more pronounced through time at El Coral, when compared to Mario. Even after one week of the restocking, the grazing effects of *D. antillarum* were evident at El Coral and Mario (Fig. 8 a, b), as *Dictyota* cover was reduced by 49% and at El Coral, and 48% at Mario. At El Coral, turf mats were significantly reduced by 43% during the first week. Other fleshy macroalgae were significantly reduced by 88% at Mario during the first week.

The reef substrate was characterized by turf algae, CCA, and pavement (Fig. 8 a, b) at El Coral by the end of the study. Turf algae, CCA, and pavement increased by two and one orders of magnitude, respectively. By the second month after restocking, most of the *D. antillarum* escaped the plots, and therefore the grazing effects at Mario were not as pronounced as at El Coral. By the end of the study, *Dictyota* spp. and fleshy macroalgal cover was reduced by 87%, respectively. The substrate at Mario was characterized by more turf algae, CCA and pavement (Fig. 8b).

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Discussion

Modern Caribbean coral reefs have been overwhelmed with benthic algae, partly due to the lack of recovery of *D. antillarum* (Lessios 2016) and the absence of other invertebrate herbivores (Francis et al. 2019; Spadaro & Bulter 2021) and larger parrotfish (rainbow, midnight, etc.). As seen in this project, the reintroduction of *D. antillarum* is an effective mitigation tool to reduce the benthic algae on coral reefs. The reduction of algae was independent of benthic composition and/or location of restocking. In just two months after reintroduction, fleshy macroalgae were reduced by 88% in cover. These grazing rates of fleshy macroalgae were higher than reported for other herbivore invertebrates (Butler & Mojica 2012; Butler & Kintzing 2016; Spadaro & Bulter 2021). The largest variation in benthic cover and algal reduction occurred with a *D. antillarum* density of 5 ind m^{-2} (Olmeda-Saldaña et al. 2021). However, a significant reduction of algae continued with two individuals per square meter. The restocking of *D. antillarum* did not significantly impact the coral cover, yet three small coral recruits were observed in one corral at El Coral and Cayo Diablo by the end of the study. Given the recruit size and duration of this study (two months), these recruits were most likely established before the experiment and covered by macroalgae. The likelihood of these coral recruits surviving has increased as they do not have to compete for space with fleshy macroalgae.

The negative relationship between *D. antillarum* abundance and fleshy macroalgal cover is not novel. Past studies have found *D. antillarum* recovery, whether natural or human-induced via restoration, greatly reduces the fleshy macroalgal abundance and positively impacts coral recruitment on a coral reef (Edmunds & Carpenter 2001; Nedimyer & Moe 2003; Macia 2007). However, fleshy macroalgae is not the dominant algal type on some modern Caribbean coral reefs. Coral reefs once characterized by turf and fleshy macroalgae, are now dominated by these

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peyssonnelid algal crusts (PAC, Edmunds et al. 2019), more specific, *Ramicrusta* in Puerto Rico (Williams & Garcia-Sais 2020). There is limited information about the distribution and ecology of most peyssonnelids, especially *Ramicrusta*. Currently in Puerto Rico, *Ramicrusta* cover is remarkably high on east coast reefs, areas like Fajardo. However, it has been progressively spreading throughout Puerto Rico (Williams & Garcia-Sais 2020). Peyssonnelids, like *Ramicrusta*, are known as 'detractors' as they do not provide suitable surface for coral settlement and might be chemically defended against the predation of herbivorous fishes (AGRRA 2020), as scrape or scar marks or predation from herbivorous fishes have not been observed in the field (Williams & García-Sais 2020). I did see scar marks on the *Ramicrusta* in corrals with *D. antillarum*. Actively restoring *D. antillarum* populations can reduce *Ramicrusta* cover by as much as 71% in two months. This study is the first report of a marine organism effectively grazing and controlling the abundance of *Ramicrusta*. These results are promising since *Ramicrusta* poses a real threat to corals and possibly other benthic organisms in Puerto Rico and other places in the Caribbean (Edmunds et al. 2019, Williams & García-Sais 2020).

All the sites in this study were characterized by a substrate cover not optimal for the settlement and coral growth before the reintroduction of *D. antillarum*. The abundance of *Ramicrusta* was exceptionally high at the Fajardo sites, and in La Parguera, sites were dominated by mostly *Dictyota* spp. and thick turf algal mats with sediments. Fleshy macroalgae, especially *Dictyota* spp. can retard growth rates of juvenile corals by outshading and abrasion (Box & Mumby 2007). The allochemicals produced by brown, fleshy macroalgae, can also introduce dangerous bacteria and inhibit coral larvae from settling (Morrow et al. 2017). Turf, which is short, filamentous, and without sediment, will not affect the settlement and survivorship of corals (O'Brien & Scheibling 2018). However, quite often, algal turf will accumulate sediments when

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there is a terrestrial source close by, or there is constant resuspension of sediments, as observed at the La Parguera sites. Birrel et al. (2005) found that coral settlement and juveniles were significantly reduced when algal turf accumulated sediments. In the Florida Keys, turf with sediment significantly inhibits coral from settling by 10 to 13-fold, compared to turf algae alone (Speare et al. 2019). By the end of this study, the substrates inside the corrals were characterized by a cleaner substrate with significantly less fleshy macroalgae, thick turf mats with sediments, and *Ramicrusta*. Pavement, crustose coralline algae, and filamentous turf algae increased from one to two orders of magnitude in the corrals at all sites. Enhancing herbivory (Francis et al. 2019), in this case, *D. antillarum* populations, resulted in more optimal reef substrate and persistence of coral reefs.

Even though *D. antillarum* were restocked to similar reef habitats (mostly dead *O. annularis* colonies), the retention of the sea urchins between the corrals and sites varied. Trying to maintain *D. antillarum* densities in the experimental plots was challenging in this study and other *D. antillarum* restoration projects (Nedimyer & Moe 2003; Miller et al. 2006). The sites with the highest retention of *D. antillarum* were at El Coral in La Parguera and Cayo Diablo in Fajardo. Two possible reasons for the higher retention at these sites could be food availability and the absence of conspecific aggressors (Sammarco & Williams 1982). The two corrals that had the most *D. antillarum* at the end of the experiment had a higher fleshy algal abundance at the beginning of the study. Sea urchins may have escaped the corrals with less fleshy algae to search for food at nearby coral heads. Williams (1979) recorded threespot damselfish to be more aggressive to *D. antillarum*, than to other sea urchins, and directly affecting the distribution of sea urchins on the reef. There were less damselfish on corals heads at El Coral and Cayo Diablo, than to the other two sites. There were at least two to four threespot damselfish, *Stegastes*

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planifrons, in each corral at Mario. *S. planifrons* are one of the most aggressive damselfish in the Caribbean, and are known to be more aggressive to conspecifics, than to predators (Kapetanaki 2008). Damselfish were noticed chasing and pecking at the spines of the reintroduced *D. antillarum*. Pieces of spines were commonly observed inside the corrals at Mario. Further studies are needed to better understand the habitat preferences of *D. antillarum*, as this may further the understanding of their distribution and improve restocking efforts.

Since 2014, *D. antillarum* settlers have been collected and lab-reared in Puerto Rico. The first major restocking of *D. antillarum* took place in 2016 on the backreef of Media Luna in La Parguera (Williams 2016). Three hundred and forty-three lab-reared *D. antillarum* were reintroduced at Media Luna, which happens to be a permanent station for the Puerto Rico Coral Reef Monitoring Program (PRCMP). Since the reintroduction, *D. antillarum* densities have continually increased from zero in 2015, to 8.0 ind 30m^{-2} in 2017 and 13.2 ind 30m^{-2} in 2019 (García-Sais et al. 2017; 2019). Resulting in the highest densities ever recorded for any permanent stations in the PRCRMP (García-Sais et al. 2017). There is evidence for positive density-dependent effects in recovery of *D. antillarum* populations (Hunte & Younglao 1988; Miller et al. 2007). I have observed many times small *D. antillarum* recruits inside the corrals with the restocked individuals. Restocked *D. antillarum* at Media Luna may be attracting the natural recruitment and local recovery of this species or the recovery may be attributed to density-dependent habitat selection (Rogers & Lorenzen 2016). Even though this study shows success, there are some limitations, such as the short duration of the monitoring. Monitoring for a longer period needs to occur to identify the long-term impacts of restocking *D. antillarum*. Additional limitations include the retention of individuals inside corrals for the study durationand *D. antillarum* behavior. Another limitation of this study was the lack of a procedural control

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(corral without sea urchins). The caging treatment may have affected the algal abundance and composition through the study. However, I do not believe that was the case because Olmeda-Saldaña et al. (2021) study was conducted during the same time and found no caging effects on algal composition and abundance. Also, there was at least one corral at each site (except at El Coral) where all the *D. antillarum* escaped during the first week of restocking. The algal composition and abundance did not change through time inside these corrals without sea urchins. Lastly, due to the natural dynamics, algae increase in abundance during the summer with higher water temperatures and light (Ferrari et al. 2012). This study occurred during late summer, when algal cover should be at its highest. Algae increased in abundance only in the control plots, not inside the corrals with the sea urchins. Given the evidence of this study and others (Olmeda-Saldaña et al. 2021, Williams and Olmeda-Saldaña 2021), te restoration of native sea urchins is a non-invasive and useful approach to aid the mitigation of algae on coral reefs. However, precaution should be taken when restoring urchin densities, as pre-mortality densities negatively affected coral spat (Sammarco 1980).

The restoration of coral reefs has taken a mostly monospecific approach, by only outplanting scleractinian corals. Many of the reefs receiving the coral outplants are overwhelmed by benthic algae, which are manually removed by divers. Protection of herbivorous fishes, such as parrotfishes, has been the focus to alleviate the algal problems on Caribbean corals reefs (Bellwood et al. 2004; Mumby 2006). However, studies have shown that herbivorous fishes are not effectively reducing macroalgae, as they tend to target more pala algae, like filamentous turf algae (Burkepile & Hay 2010; Briggs et al. 2018). Not all herbivores are the same in reducing algae, as they have different behavioral and morphological

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traits which affect what they consume and the efficiency of their grazing (Carpenter 1986; Strait et al. 2015). As seen in this study, *D. antillarum* effectively reduces *Dictyota* and other fleshy algae, but will not consume *Halimeda*, another prevalent alga on coral reefs. The Caribbean king crab, *Maguimithrax spinosissimus*, may fill this niche, as they effectively consume *Halimeda* spp. and fleshy macroalgae (Spadaro & Butler 2021) . If restocking *D. antillarum* is not an option, other sea urchins, specifically *Echinometra viridis* and *Tripneustes ventricosus* (Francis et al. 2019), may also be used to control *Ramicrusta* and other algae (respectively). *E. viridis* have been proven to be as efficient at grazing algae as *D. antillarum* when at higher densities (Sangil and Guzman 2016; Kuempel et al. 2017). In the field, I have observed lower abundance of *Ramicrusta* at sites where *E. viridis* were prevalent (>5 ind m^{-2}). Therefore, to effectively reduce benthic algae, protection and/or restoration should take a more diverse approach by enhancing a mixed herbivore assemblage, such as crabs, fish and sea urchins (Lubchenco & Gaines 1981; Burkepile & Hay 2008).

Acknowledgements

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Figures

Figure 1. A map of the restoration sites, Cayo Diablo and Los Lobos in Fajardo and El Coral and Mario in La Parguera, Puerto Rico.

Figure 2. Photograph of a) a corral at Los Lobos, b) restocked *Diadema antillarum* inside a corral at Cayo Diablo that was dominant in *Dictyota* spp. and *Ramicrusta* spp., c) *Diadema antillarum* one month after restocking event in Fajardo, and d) top view of a corral at Los Lobos, Fajardo.

Figure 3. Mean abundance of *Diadema antillarum* inside the corrals through the monitoring at sites in Fajardo (Cayo Diablo and Los Lobos) and La Parguera (El Coral and Mario), Puerto Rico. The bars denote standard errors.

Figure 4. Principle coordinate analysis (PCO) of the benthic composition between control and experimental plots (corrals) before (left) and two-months (right) after the reintroduction of *Diadema antillarum* in Fajardo, at Cayo Diablo (a) and Los Lobos (b), Puerto Rico. Plots 1-6 were the experimental corrals with *D. antillarum*, and Plot 7 was the control. CCA= Crustose coralline algae.

Figure 5. Mean cover of the benthic substrate inside the corrals with *Diadema antillarum* through the sampling time at a) Cayo Diablo (n=164) and b) Los Lobos (n=126) in Fajardo, Puerto Rico. The bars denote standard errors.

Figure 6. Photographs of a permanent quadrat before (left) and two months (right) after the restocking of *Diadema antillarum* in Corral 5 at Cayo Diablo, Fajardo. Red circles indicate *Ramicrusta*.

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Figure 7. Principle coordinate analysis (PCO) of the benthic composition between control and experimental plots (corrals) before (left) and two-months (right) after the reintroduction of *Diadema antillarum* in La Parguera, at El Coral (a) and Mario (b), Puerto Rico. Plots 1-6 were the experimental corrals with *D. antillarum*, and Plot 7 was the control. TWS= Turf with sediment and CCA= Crustose coralline algae.

Figure 8. Mean cover of the benthic substrate inside the corrals with *Diadema antillarum* through the sampling time at a) El Coral (n=197) and b) Mario (n=189) in La Parguera, Puerto Rico. The bars denote standard errors. TAS: Turf algae with sediment.

Figure 1

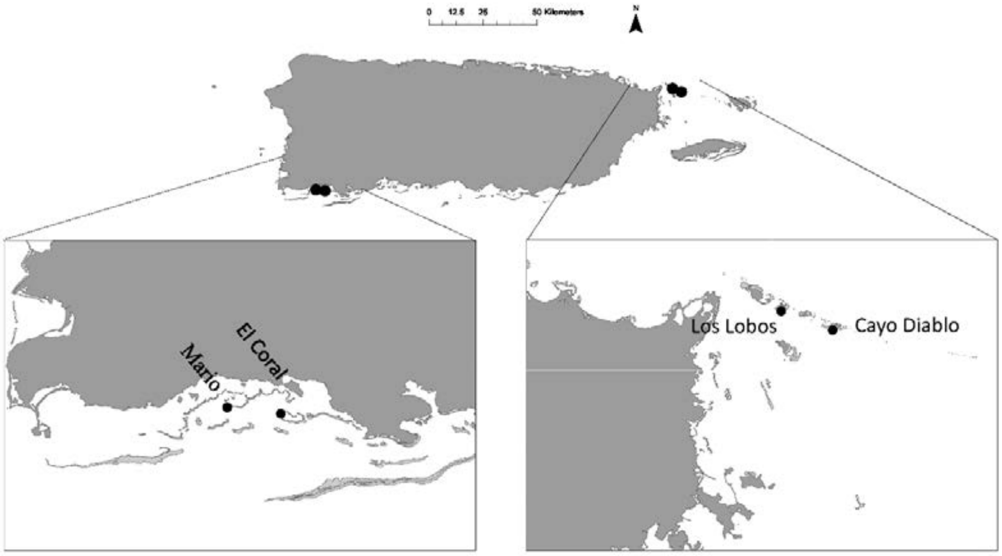

Figure 2

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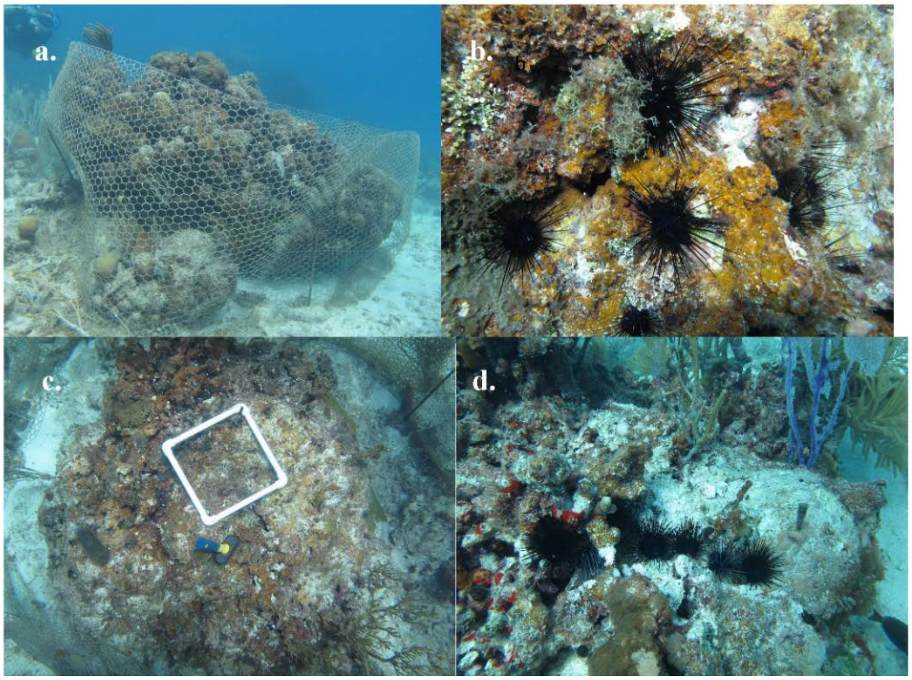

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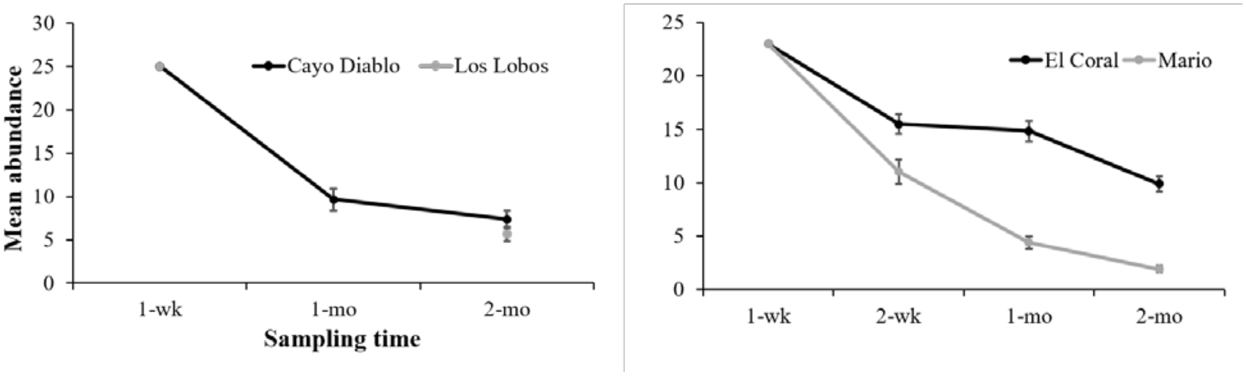

Figure 4

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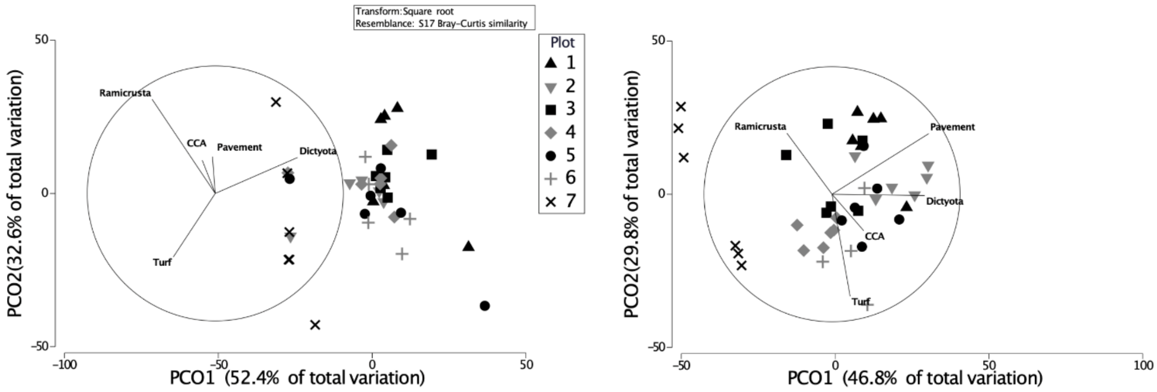
Cayo Diablo



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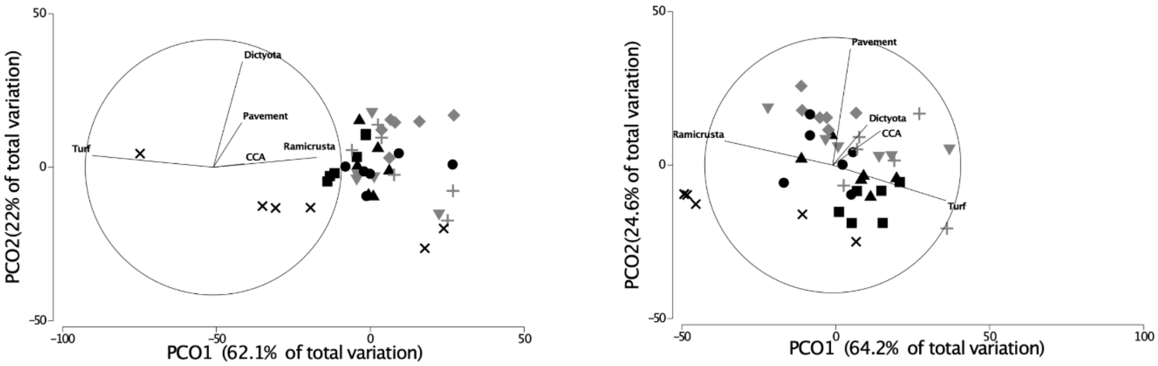

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Figure 5

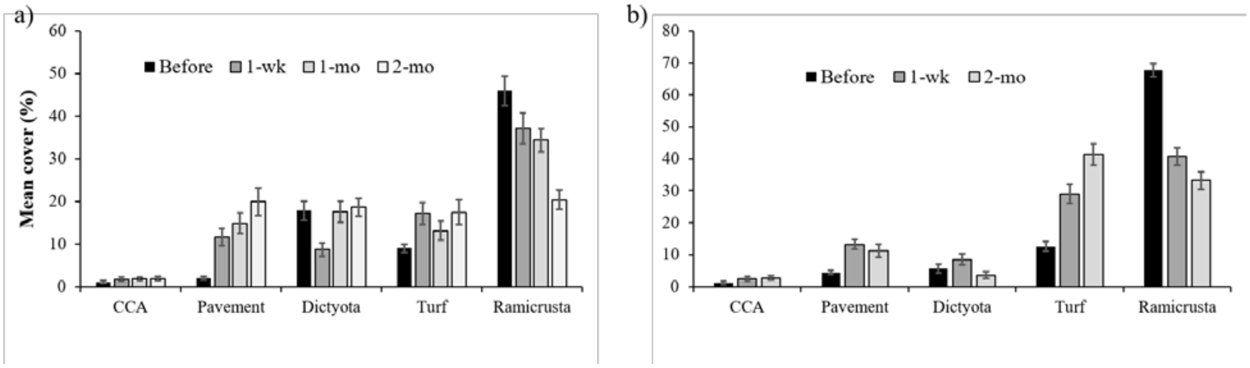

Figure 6

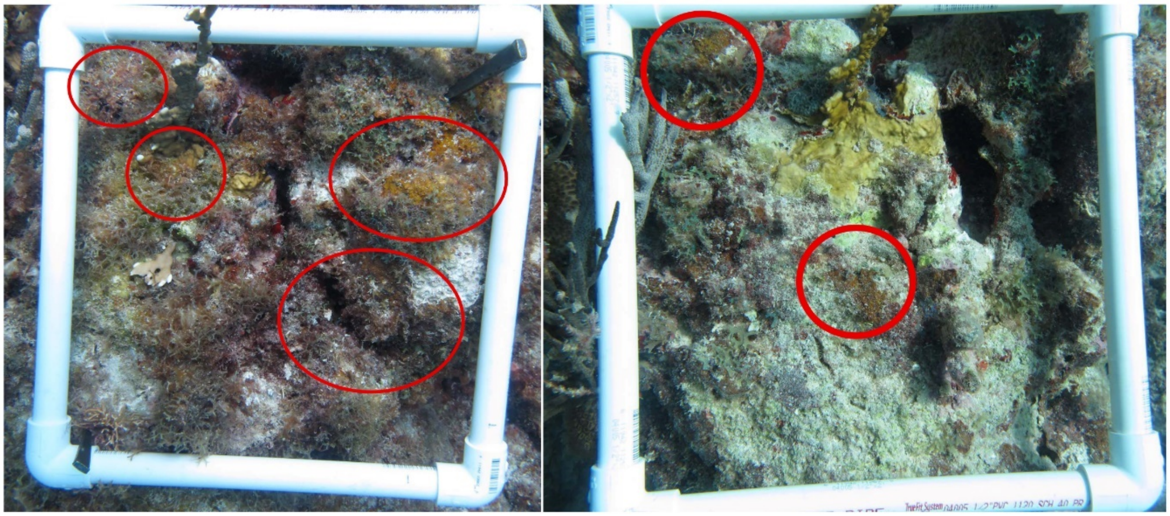

Figure 7

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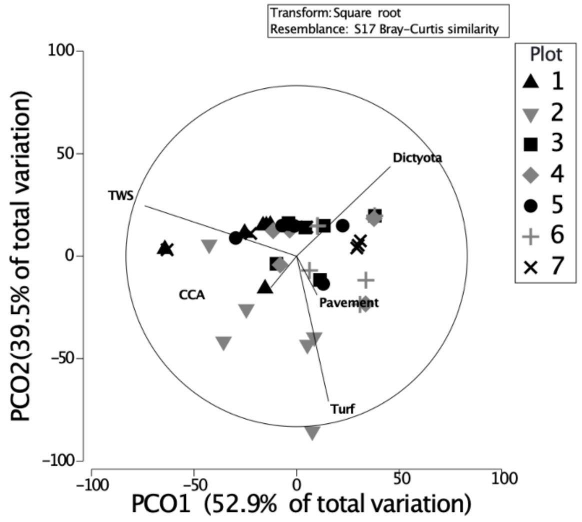
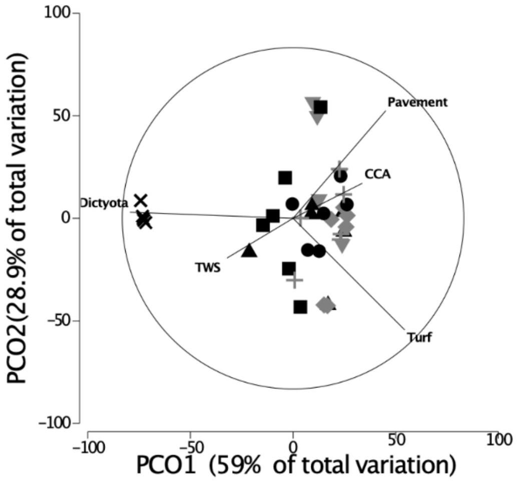
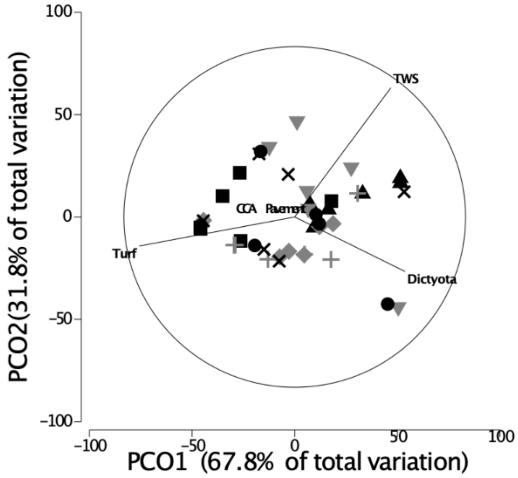
El Coral





Mario



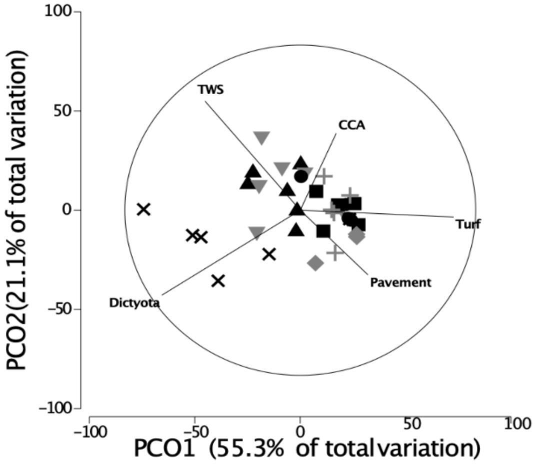


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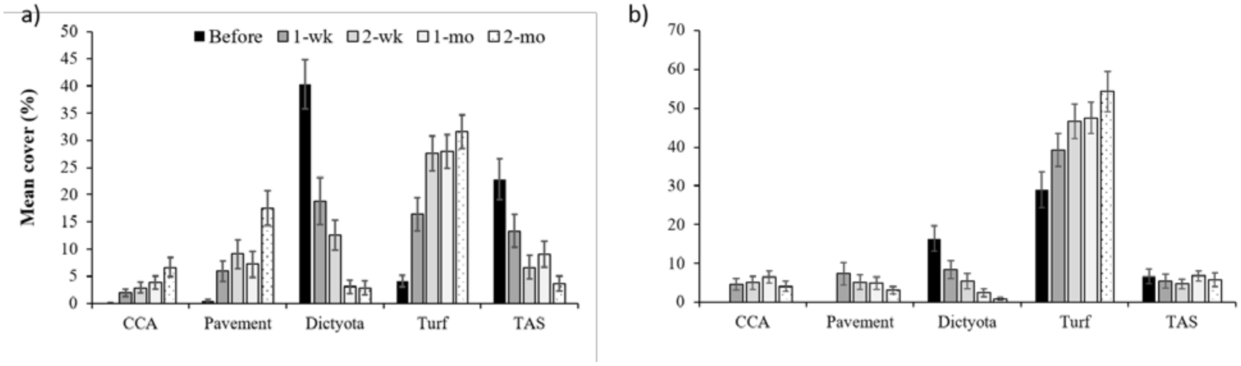
Figure 8



Supplementary files

Table S1. The results of the three-way Permutational Analysis of Variance (PERMANOVA) test to examine the benthic composition change between sites (Si), plots (Co), and sampling time (Ti) at Fajardo, Puerto Rico. Plots were nested within sites and *Diadema antillarum* (Di) abundance was the covariable.

Fajardo	**df**	**SS**	**MS**	**Pseudo-F**	**P(MC)**
Di	1	18567	18567	10.34	0.001
Si	1	20669	20669	2.87	0.042
Ti	3	37329	12443	4.86	0.006
Co(Si)	12	84142	7011.8	11.72	0.001
SixTi	2	4106.9	2053.5	2.29	0.037
TixCo(Si)	29	25777	888.85	1.49	0.009

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Table S2. The results of the three-way Permutational Analysis of Variance (PERMANOVA) test to examine the change in *Ramicrusta* cover between sites (Si), corrals (Co), and sampling time (Ti) at La Parguera, Puerto Rico. Plots were nested within sites and *Diadema antillarum* (Di) abundance was the covariable.

Ramicrusta	**df**	**SS**	**MS**	**Pseudo-F**	**P(MC)**
Di	1	1.5294	1.5294	0.27	0.604
Si	1	93.841	93.841	5.76	0.038
Ti	3	227.39	75.795	11.07	0.014
Co(Si)	12	190.62	15.885	4.97	0.001
SixTi	2	10.705	5.3523	1.20	0.3
TixCo(Si)	29	128.61	4.4348	1.39	0.109

Table S3. The results of the three-way Permutational Analysis of Variance (PERMANOVA) test to examine the benthic composition change between sites (Si), plots (Co), and sampling time (Ti) at La Parguera, Puerto Rico. Plots were nested within sites and *Diadema antillarum* (Di) abundance was the covariable.

La Parguera	**df**	**SS**	**MS**	**Pseudo-F**	**P(MC)**
Di	1	25224	25224	4.82	0.025
Si	1	54363	54363	4.98	0.01
Ti	4	1.01E+05	25268	6.18	0.001
Co(Si)	12	1.29E+05	10788	10.71	0.001
SixTi	4	14013	3503.4	2.01	0.03
TixCo(Si)	44	75207	1709.2	1.70	0.001