Seeding reefs with *Diadema antillarum* to enhance coral recovery in Puerto Rico

2014 Annual Report

Introduction

Structure dynamics of coral reefs are intricate and continually changing, many times due to disturbances. Before the 1980's, Caribbean reefs were predominantly characterized by reef-building coral species, with an absolute live coral cover of 55% (Gardner et al. 2003). Over the past three decades coral reefs in the Caribbean have change dramatically (Ginsburg 1994, Hughes 1994, Jackson 1997). Abundance of reef-associated organisms, especially corals, have suffered a massive decline due to cumulative factors such as, hurricanes, disease outbreaks, bleaching, pollution, and overfishing (Bythell and Sheppard 1993, Bythell et al. 1993, Littler et al. 1993, Hughes 1994, Kramer et al. 2003).

One of the most dramatic shifts in community structure occurred after the massive die-off of *Diadema antillarum*, a keystone herbivore. The 1983-1984 mass mortality of *D. antillarum* occurred throughout the Caribbean basin and was the most extensive and severe die-off ever recorded for a marine invertebrate (Lessios 1995). Before 1983, the presence of this organism was common (13-18 ind/m^2) on coral reefs in the in Puerto Rico (Bauer 1980, Vicente and Goenaga 1984). Adult *D. antillarum* play an important role in structuring coral reef communities by controlling algal abundance (Carpenter 1981, Carpenter 1986, Carpenter 1990a, Carpenter 1990b, de Ruyter van Steveninck and Bak 1986, Odgen et al. 1973, Robertson 1987, Sammarco 1982), productivity (Williams 1990) and is one of the principal agents of bioerosion on reefs (Lidz and Hallock 2000, Bak et al. 1984, Scoffin et al. 1980). It has also been found that *D. antillarum* is a commensal for many organisms such as: juvenile fish, crustaceans, and echinoderms (Randall 1964, Steiner and Williams 2005). After the massive die-off, populations were drastically reduced by 95-100% in many Caribbean locations (Lessios 1995) and at the same time fleshy macroalgal cover increased between 100% and 250% (Phinney et al. 2001). The absence of *D. antillarum* did not only influence benthic algal productivity of coral reef communities, but it also impinged on the settlement of sessile recruits, such as, corals and the growth of adult corals.

Presently, the recovery of *D. antillarum* has been slow and even absent at many locations in the Caribbean. In Puerto Rico, there has been a modest recovery in the population of *D. antillarum* (Mercado-Molina et al. 2014); however densities are still far below pre-mass mortality numbers. Larval mortality and recruitment have been suggested as the main factors regulating the adult population size of *D. antillarum* (Karlson and Levitan 1990). In Puerto Rico, upstream sources of *D. antillarum* “settlement-ready” larvae are available (Williams et al. 2010), therefore larval supply and survival does not seem to be an inhibiting the recovery. Recruitmentlimited processes, such as post-settler and/or recruit mortality may be regulating the population dynamics of *D. antillarum* in Puerto Rico.

Self-reinforcing negative feedbacks (no herbivores) will drive reefs towards a coral-depauperate stable equilibrium (Roff and Mumby 2012). Areas, especially in Jamaica have seen positive feedbacks from the recovery of *D. antillarum*. *D. antillarum* recovery has been coupled with the decrease of macroalgal cover on the shallow-water reefs of Jamaica (Woodley 1999, Aronson and Precht 2000, Cho and Woodley 2002, Bechtel et al. 2006, Carpenter and Edmunds 2006). In addition, the survival of juvenile and adult corals has increased with *D. antillarum* recovery (Edmunds and Carpenter 2001, Idjadi et al. 2010).

Study Objectives

Short-term goals were:

1) To increase populations of *D. antillarum* at a reefs in La Parguera and at restored reef in Guayanilla, Puerto Rico
2) Monitor grazing effect and survival of settlers and transplanted juveniles

The long-term goal of this project is to enhance coral reef resiliency, such as increasing (coral recruitment, survival, and growth and decreasing algal abundance by introducing herbivorous benthic organisms.

Materials and methods

This project followed the same methodology outlined in Williams et al. (2010, 2011) settlement studies. Williams et al. (2010, 2011) observed that shelf-edge sites, Old Buoy and El Hoyo, were "hot spots" for *D. antillarum* settlement (max of 1,064 ind m^{-2}). The supply of *D. antillarum* settlers for this restoration experiment was collected at the Old Buoy (Fig. 1). The depth of the site ranges from 18-21 meters. The reef substrate is fairly flat with relatively low coral cover and diversity. Historically, there have been few adult *D. antillarum* recorded at both of these sites (<0.01 ind m^{-2}). Williams et al. (2010, 2011) observed that the settlement of *D. antillarum* was highest during summer months. Therefore, ten mooring lines were placed at Old Buoy on June 2, 2014. Two cement blocks anchored each of the

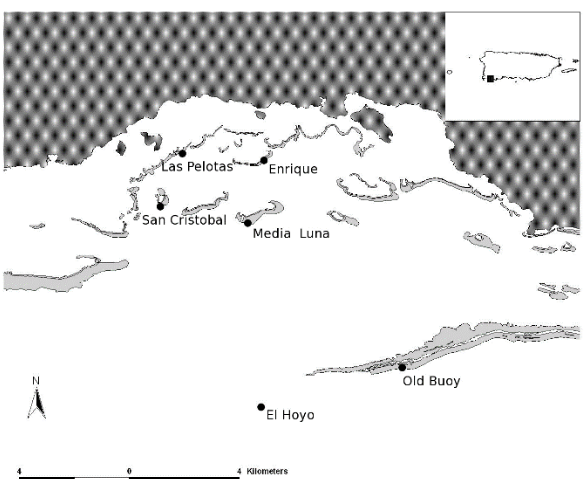


Fig. 1 Settlement sites in La Parguera, Puerto Rico

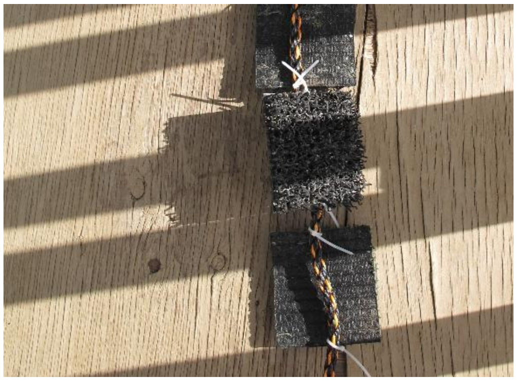


Fig. 2 Astroturf or artificial turf used as settlement

mooring buoys. The cement blocks were placed in the middle of a sand channel, and not impacting the corals and reef on either side. The buoys were located at 6 m of depth and did not represent any navigational hazard. On each mooring line, a rope containing 20, 8 × 8 cm pieces of artificial turf (Fig. 2) was positioned between 7-10 meters. The rope was detached and placed in a dry bag. Settlement plates were replaced every month until October 2014 (total of four samplings) and were brought back to the laboratory to analyze. The mooring lines were removed during our collection in October.

Culturing Diadema antillarum settlers

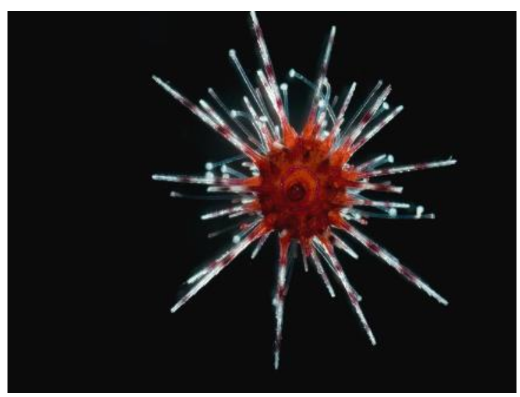


Fig. 3 Diadema antillarum settler

Settlers (Fig. 3) were picked off each settlement plates, counted and transferred to 10-gallon tanks and then switched to a closed filtering system during the end of August. This helped reduce the sediment and other larvae from entering in the tanks. Tanks were cleaned once a week and one third of the water was replaced from stocked seawater (seawater stored in a dark place for a week). Water quality measurements were recorded every week. These measurements included salinity, pH, temperature, nitrate, and ammonium. Brown and red algae (*Padina* and *Laurencia* spp.) were collected, washed with freshwater, inspected for micropredators (worms and crabs), and placed in the tanks. Algae were collected once a week. Settlers were transferred to wet tables once they reached a size of 5 mm (Fig. 4). Juvenile damselfish were placed in each wet table with the *D. antillarum* settlers. The water movement and/or pheromones from the fish may stimulate protective behavior of *D. antillarum* settlers, which might help prepare them for life on a coral reef (Martin Moe, pers comm.). Tables were cleaned and algae were collected and placed in each table every three to four days. Fish were fed with dried pellets every other day. *D. antillarum* juveniles were transplanted to the reef once they reached a test size of at least 3 cm.

Fig. 4 Wet tables at Marine Science Department

Transplantation

D. antillarum juveniles were transplanted to three different reefs (Fig. 5), backreefs of Media Luna (N°17.93948, W°-67.05070) and Margarita reef (N°17.91964, W°-67.12848) in La

Parguera and a forereef in Guayanilla, Matthew's reef (N°17.96164, W°-66.75705).

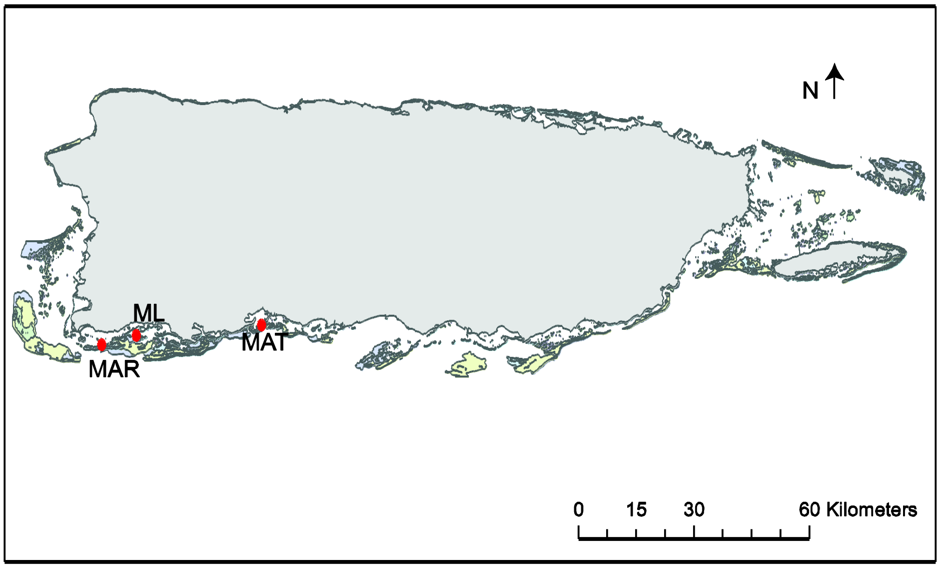


Figure 5 Map of sites where Diadema antillarum were transplanted. ML: Media Luna, MAR: Margarita and MAT: Matthew's reef

At each location, half of the transplanted juveniles were placed in cages, while the other half placed freely on the reef. This allowed for comparison of survivorship in the caged versus uncaged juveniles. The cages were placed in areas, barren of corals and benthic organisms and they will were secured to the sea floor with rebar.

Media Luna reef

The backreef of Media Luna is a structurally-complex reef, comprised of mostly dead *Orbicella annularis* colonies. The dominant benthic substrate on the reef at Media Luna is fleshy microalgae, particularly *Dictyota* spp. The density of *D. antillarum* on this reef is less than 0.05 ind m^{-1}. We did a thorough search and did not locate any juvenile *D. antillarum*.

Matthew's reef

In Guayanilla, Puerto Rico, there have been several large vessel groundings over the past 20 years that have had significant impact to coral. On December 15, 2009, the vessel LNG/c Matthew grounded on a coral reef, now named Matthew's reef. Restoration efforts at this reef consisted of gluing broken coral heads from the grounding and reseeding corals, like *Acropora cervicornis* from nearby nurseries. The reef complexity is relatively flat when compared to the backreef of Media Luna. The transplanted *A. cervicornis* colonies contribute to most of the rugosity at this reef. The density of adult *D. antillarum* at this site is low (<0.05 ind m^{-1}).

Margarita reef

Finally, the last transplantation site was located at Margarita reef, which is the longest mid-shelf reef of La Parguera. Coral nurseries of acroporids are located in the western backreef at Margarita reef. The backreef is mainly composed of many small patch reefs, dominated by dead *Orbicella* spp. There were a couple of tall dead *O. annularis* colonies with high densities of adult and young adult *D. antillarum* (~2 ind m^{-2}). On these colonies, fleshy macroalgae were low and crustose coralline algae were high. The larger patch reefs had a relatively low *D. antillarum* density (<1 ind m^{-2}). Densities were comparable to the backreef of Media Luna.

Results

Settlement

A total of 1,346 settlers were collected during the four month collection. As seen in Figure 6, settlement of *D. antillarum* varied among the sampling months, with a peak in settlement in July 2014.

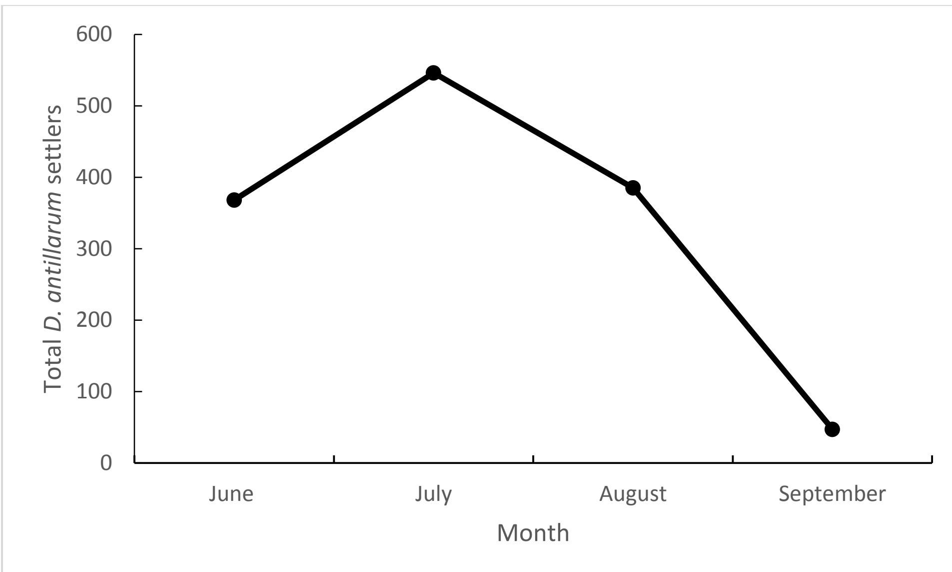


Fig. 6 Total amount of D. antillarum settles collected during each month in 2014.

The size of the settlers ranged from 0.4 mm to 1.0 mm. There were settlers that were larger than 1 mm in test size collected in July and August. These settlers may have settled the month before on the rope and migrated to the plates. Approximately, four percent of the settlers died either during transporting them back to lab and/or when sampling through settlement plates.

Survivorship and growth

It was hard to measure survivorship during the early stages of culturing because of the size of the settler. During July and August, high settler mortality (~90%) occurred. During this time tanks were not on a closed-filtering system, and a lot of sediment and other settlers, such as worms were abundant in the tanks. We move to a closed-filtering system at the end of August. The survival of juveniles increased by 54% after moving to the closed-filtering system. Water quality measurements were normal during all samplings. However, salinity fluctuated from 34‰ to 36‰. Six hundred milliliters of distilled water was added to each tank when salinity was greater than 35‰.

On August 12, 2014, there were a total of 369 settlers in the 10-gallon aquariums and the first set (17 in total) of settlers was transported to the larger wet tables. During this time the smallest individual in the 10-gallon tanks was measured at 8.37 mm, while the largest was recorded at 13.76 mm in test diameter. The last set of settlers transferred from the 10-gallon aquariums to the wet tables was on November 20, 2015 and the total amount of settlers/juveniles in the wet tables at this time was 200 individuals. On January 16, 2015 the settler/juvenile count dropped to 191 and then to 183 individuals on February 9, 2015. The survivorship of settler/ juveniles in the wet tables was 92% during a seven month period. Settlers with a test diameter size less than 5mm were more vulnerable than larger individuals. We plan on building a bio-filter and connecting it to the water system to help eliminate any pathogens, sediment and/larvae from entering the tanks. We hope that survivorship of settlers will increase with cleaner water.

Bak (1985) noted that the average weekly growth rate of a settler or juvenile is 1.1 mm wk^{-1}. However, we measured settler growth to be higher in our rearing experiments (ranging from 4 mm mo^{-1} to 5 mm mo^{-1}). Some individuals grew faster than others; it seemed to be space dependent. Therefore, settlers would take a longer time to grow if there were more individuals in the tank. The largest individual recorded during February 18, 2015 was 4 cm in test diameter. I observed that a juvenile urchin needs about 3 gallons and space for optimal growth, for example, 50 juvenile urchins are sufficient for a 150 gallon tank.

Miscellaneous comments

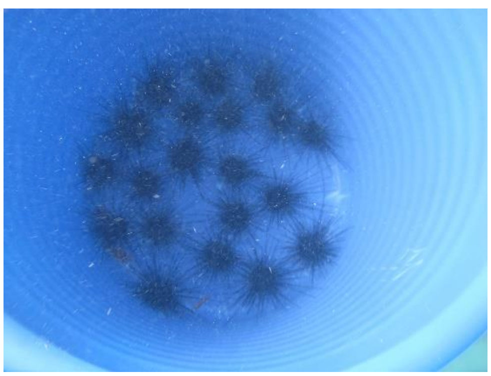
- Special care (reduce sediment, and other possible micropredators) should be taken when settlers are small (< 5mm in test diameter). They are vulnerable at this stage.
- *D. antillarum* prefer brown and red algae, *Stypopodium* sp., *Laurencia* sp. and *Gracilaria cuneata*. They would consume these algae first and *Dictyota* sp. would be the last algae consumed.
- Juvenile (> 1 cm) *D. antillarum* do not seem that vulnerable to high sediment load compared to settlers. There were many times when the water system was shut off. During these instances water from the holding tanks would drain fully into the tables, including the silt on the bottom of the holding tanks. This seemed to not bother the urchins.
- Tanks need to be cleaned every couple days. Larvae from the water and organisms from the holding tanks would colonize in the wet tables. These organisms, such as crabs and polychaetes could be potential predators of the smaller juveniles. Rocks in the wet tables should also be cleaned every two weeks.

Transplantation

Media Luna, La Parguera

The first transplantation occurred on the backreef of Media Luna in La Parguera on February 18, 2015. Twenty *D. antillarum* were placed freely on the reef, while 20 juveniles were placed in three separate cages. Each cage had a surface area of about 1 m^2 and will be made out of ¼" plastic mesh and PVC. For the uncaged urchins, 10 were place on one *O. annularis* colony and 10 more were placed on another small colony about 10 feet away. Both *O. annularis* colonies were marked with flagging tape. Cages were removed a week later (February 25, 2015) at night.

Pictures of Media Luna Transplantation



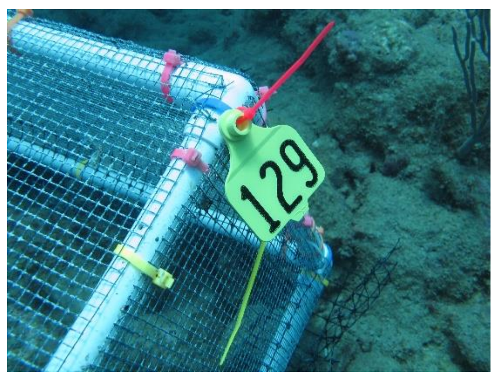
More than half of the juveniles placed freely on the reef were observed two days after the transplantation. There were no signs of predation (no spines and/or dead urchins). Therefore, given the rugosity of the site it is possible that some of the juveniles were hiding in crevices. We revisited the site a week later (February 25) at night and marked the distance the urchins traveled from the original area. The farthest distance a juvenile traveled in a week was 7 m. We removed the cages after marking the distance of the juveniles. We returned to the site on March 1, 2015 and could not locate any of the juveniles due to the visibility of the water.

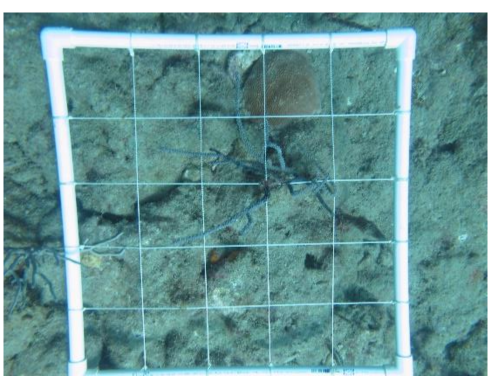
Matthews's reef, Guayanilla

On March 15, 2015, 46 *D. antillarum* were placed in 5 separate cages, while 23 young adults were placed on two small patch reefs (total 69). Each cage had a surface area of about 1 m^2 and will be made out of ¼” plastic mesh and PVC. Cages were tagged and we measured the benthic cover and coral recruits with one photo-quadrat inside and outside each cage (1 m x 1 m). Photo-quadrats will be taken again when cages are removed.

Pictures of Matthew’s reef Transplantation








We returned less than a week later (March 20th) to monitor the survivorship of the young adults. One *D. antillarum* was missing from the cages and one escaped the cage (98%

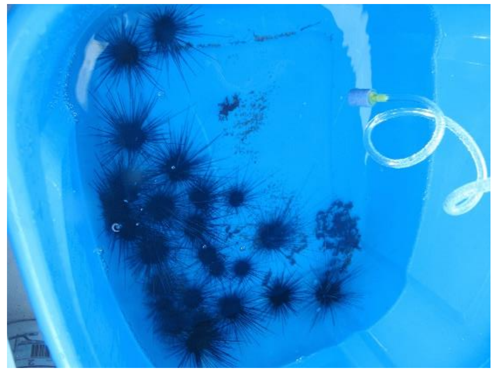
survivorship). That urchin was replaced back in the cage. We could not locate the ones that were set free. Again, we did not see any spines or body parts on the reef substrate. In addition, the reef substrate on the small patch reefs did not looked grazed.

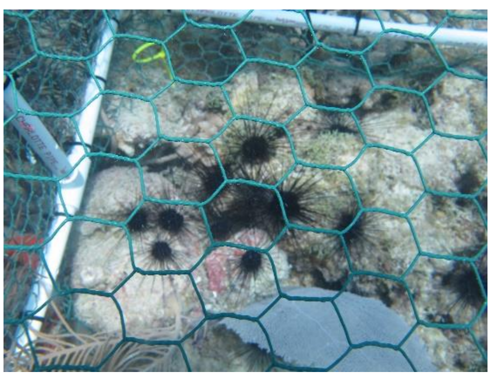
In April, the cages were removed and the urchins were placed in “corrals”. Corrals were made of nylon mesh and were meant to keep the urchins in one place, while letting other organisms, like fish, to enter. However, after returning one month later to monitor, we could not determine if the urchins had escaped or been eaten. There were no spines or tests to indicate that there had been any predation. Future work will focus on creating better corrals that initially have a top so the sea urchins cannot escape and to keep predators out.

Margarita reef, La Parguera

54 *D. antillarum* were transplanted in three separate cages on March 18, 2015. The cage dimensions placed on Margarita reef (5 ft. long x 2 ft. wide x 2 ft. tall) were different from the cages placed on Media Luna and Matthew’s reef (3 ft. x 3 ft. x 1ft.). Fifteen *D. antillarum* were placed a small patch reef located right next to the coral nurseries. The patch reef was marked with tape and cages were marked with tags. We measured benthic cover and coral recruits with two photo-quadrats inside and outside each cage (1 m x 1 m).

Pictures of Margarita Transplantation





We returned less than a week later (March 24th) to monitor the survivorship and location of the transplanted young adults. None of the freed individuals were located. We did not see any spines or body parts on the reef substrate. Many of the caged urchins escaped (about 17) and were located within 5m of the cages. The urchins were recollected and placed back in the cages. The netting was secured at the bottom of each cage. The escaped sea urchins were hard to identify because they were well hidden in crevices.

Lessons Learned

- Settlers should be grown to at least 4 cm in test size. Any size less than 4 cm may make them more vulnerable to predators.
- Survivorship may be density-dependent, therefore place many on a reef at one time.
- Survivorship of transferred individuals seemed to increase on more rugose reefs.
- Urchins should be transferred to reefs during dusk or at night.
- Caged urchins that escaped seem to adapt quicker to the reef environment than those which were set free.
- Corrals should be used instead of cages. Urchins should be enclosed in the corral for a month and then remove the cover. Predation will be easier to monitor when using the corrals.
- For monitoring purposes, night dives might improve in the identification of these small urchins on the reef substrate.

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