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Abstract

      In January 1983, a waterborne pathogen beginning near the Atlantic Panama Canal entrance caused history's largest recorded marine animal die-off, wiping out 95-99% of *Diadema antillarum* populations throughout the Caribbean. *D. antillarum*, the long-spined sea urchin, is a keystone herbivore in coral reefs and its decreased densities have caused many reefs to suffer macroalgal phase shifts. Modest recovery of this species has been documented in some Caribbean locations, but reefs in Guna Yala, Panama continued to experience population decline. This study investigates density, test size, and substrate preferences of *D. antillarum* in three shallow coral reef areas to update the species' recovery in Guna Yala. Over the total 1,080 m^2 of reef surveyed, *D. antillarum* was present at a mean density of 0.11 individuals m^{-2}. This is only 3.03% of the 1982 pre-mortality density for the area, but is a slight recovery from the 2015 density of 0.03 individuals m^{-2}. This study was the first to document test sizes in Guna Yala in 24 years and did so at novel reef sites. Test sizes ranged from 8-62.5 mm with a mean of 38.85 mm, and the presence of 6 juveniles (test size<2 cm) indicates a level of recruitment. Out of the total 119 urchins counted, 94 individuals were located under dead coral skeletons covered in algae. There were no significant relationships ($p<0.05$) between urchin test sizes and the type of substrate they resided on (within live coral, under live coral, under dead coral, or under rocks), suggesting that a factor other than size determines where *D. antillarum* are located within a reef, and that *D. antillarum* in Guna Yala could potentially aid in phase shift reduction due to their exhibited preference for algae-covered surfaces. This study demonstrated consistent average densities, test sizes, and substrate preferences between the three sites, indicating that the different sites are experiencing similar population recovery phenomena despite their varying observed characterizations. The consistent results between sites bode well for this study's representation of *D. antillarum*'s current status in Guna Yala reefs.

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Introduction

Coral reefs

Warm-water coral reefs are the world's most biodiverse marine ecosystems, occupying less than 0.1% of the ocean floor, yet providing habitat for at least 25% of all known marine species-- with many new organisms still to be discovered (Ahmadia et al. 2012; Fisher et al. 2015). Coral reefs form through hundreds and thousands of years of calcium-carbonate structure buildup by reef-building corals that are the base of coral reef ecosystems (Hoegh-Guldberg et al. 2017). Corals are marine invertebrate animals that live in symbiosis with their photosynthetic algae that provide corals with energy and nutrients to survive (Hoegh-Guldberg et al. 2017). The algae growing on corals, along with zooplankton, bacteria, and marine plants, form the primary productivity base of the coral reef food web (Graham et al. 2017). Herbivorous echinoderms and reef fishes form the main primary consumer populations (Graham et al. 2017).

Healthy coral reefs provide a number of invaluable ecosystem services, such as protecting coasts from storm damage, providing promising bioprospection opportunities, and sustaining livelihoods for fishing communities worldwide (Spillman 2011). The benefits of coral reefs are estimated to extend to over 500 million people in at least 90 different countries (Hoegh-Guldberg et al. 2017). Despite reefs' incredible value and biodiversity, anthropogenic disturbances have impaired many coral reef ecosystems by damaging fish populations and creating ocean conditions unfit for coral survival (Spillman 2011).

Like many ecosystems, coral reefs are subject to the direct impacts of pollution and physical damage by human activities; Burke et al (2011) outlines some of these main contributors. With over 275 million people living within 10 km of the coast and 30 km of a coral reef, it is not surprising that boats, both tourist and local, contribute raw sewage, fuel leaks, invasive species, and other contaminants to reefs. Many boat-related damages such as anchors and propeller destruction take corals decades to recover from. Oil spills from on and offshore drilling cause coral distress, usually resulting in mortality. Human activities such as coastal development and activities far inland also pollute coral reefs in the form of sediment runoff that weakens corals and blocks their photosynthetic algae symbionts from receiving sunlight. Sediment and nutrient pollution also cause eutrophication, the phenomenon by which excess nutrients such as nitrogen and phosphorous cause phytoplankton and algae to thrive at levels that are unhealthy for the reef ecosystem. These excess nutrients can cause algae to overgrow and outcompete corals, thus reducing overall coral cover. Eutrophication can also cause phytoplankton blooms that block sunlight from reaching photosynthetic reef organisms.

Because coral reefs are so productive and full of life, they are prone to overexploitation and overfishing practices. "Fishing down the food chain" is a phenomenon occurring in many reefs wherein the fishing industry depletes predatory fish populations of higher trophic levels, and then switches to herbivorous fish species of a lower niche on the trophic pyramid (Burke et al. 2011). In many of the world's coral reefs, this has occurred to the point where herbivorous fish are overfished, causing an overabundance of their main food source: algae on corals (Burke et al. 2011). Many Caribbean coral reefs are experiencing phase shifts to a 'coral depauperate' state, characterized by high macroalgae and sponge cover on corals due to the lack of herbivorous species to keep these populations under control (Mumby 2009). Excess nutrients, hurricanes, and coral disease can also contribute to the occurrence of macroalgal phase shifts (Mumby 2009). Reefs that have experienced phase shifts have lower coral survival and subsequently less biodiversity than reefs in the 'coral dominated' state (Mumby 2009).

A lesser-known but very active threat to coral reefs is the presence and rapid spread of pathogens. Though pathogens are a natural part of any ecosystem, because coral reefs are facing so many other threats, their ability to fight and coexist with pathogens is reduced and they are therefore more susceptible to disease (Burke et al. 2011). Diseases caused by both abiotic and biotic factors have been detected in many species of tropical marine algae, plants, invertebrates, and vertebrates (Peters 2015). For example, the white and black band coral diseases have already altered Caribbean reef ecosystems through rapidly spreading and killing coral (Burke et al. 2011).

Diadema antillarum characteristics

The black, long-spined sea urchin *Diadema antillarum*, once a ubiquitous organism on Caribbean reefs, is a victim of a waterborne pathogen and whose subsequent reduced density is associated with macroalgal phase shifts (Idjadi et al. 2010). *D. antillarum* is a member of the marine invertebrate Phylum Echinodermata, which has five different classes: Asteroidea, Ophiuroidea, Echinoidea, Holothuroidea, and Crinoidea (Pawson 2007; Chiappone et al. 2013). *D. antillarum* is in the Class Echinoidea, which contains 800 different sea urchin, sand dollar, and heart urchin species (Pawson 2007; Chiappone et al. 2013). The sea urchins in this class are split into two groups, the "irregular" urchins with bilateral symmetry, and the "regular" form urchins with obvious radial symmetry, spherical bodies, and spines all of similar length (Pawson 2007).

D. antillarum is a regular urchin characterized by its large body, long, noxious spines that can be up to 30 cm long, and its tube feet arranged in arcs of three on the underside of its body (Pawson 2007; Randall et al. 1964). It inhabits tropical coral reefs, rocks, mangrove roots, *Thalassia* beds, and sand flat habitats throughout the west Atlantic from Florida to Bahia, Brazil (Lessios 2016). *D. antillarum* has been reported living at depths of 400 m, but it prefers to inhabit shallow depths of 1-10 m (Ogden and Carpenter 1987). This species is most abundant in reef habitats, where it tends to group by size and gender for the purpose of having more spines together for mutual protection from predators (Ritz 2008; Randall et al. 1964). *D. antillarum* is also highly mobile, keeping to sheltered crevices during the day and emerging at night (Randall et al. 1964).

D. antillarum is a generalized herbivore that grazes on coral substrate, removing plant material and bits of coral that it excretes, contributing to bioerosion (Ogden and Carpenter 1987). Other reef herbivores such as parrotfish (Scaridae), damselfish (Pomacentridae), and the reef urchin *Echinometra viridis* are *D. antillarum*'s largest competitors in reef ecosystems. *D. antillarum* has been observed attacking *E. viridis* individuals intruding on its grazing areas (Ogden and Carpenter 1987). Fifteen different fish species have been observed predating on *D. antillarum*, in the triggerfish (Balistidae), jack (Carangidae), porcupinefish (Diodontidae), wrass (Labridae), boxfish (Ostraciidae), porgy (Sparidae), and pufferfish (Tetraodontidae) families (Randall et al. 1964). Two gastropods of the genus *Cassis* predate on *D. antillarum*, as well as the spotted spiny lobsters *Panulirus guttatus* and *Panulirus argus* (Randall et al. 1964; Kintzing and Butler 2014). In *P. guttatus*' presence, *D. antillarum* consume less algae and exhibit hiding behavior, but act no different in the presence of *P. argus* (Kintzing and Butler 2014).

D. antillarum become sexually mature at test diameters of 3-6 cm (Ogden and Carpenter 1987). They exhibit asynchronous broadcast spawning, wherein females release 1 million eggs every new moon year-round (Ritz 2008; Lessios 2016). Not a lot is known about the recruitment process and *D. antillarum*'s planktonic stage, but juveniles (<2 cm test diameter) can be observed

within 1-2 months of settling on the bottom (Randall et al. 1964; Ogden and Carpenter 1987). Juvenile *D. antillarum* exhibit cryptic behavior and grow at rates of 3.5-8 mm per month (Lessios 2016; Randall et al. 1964; Bauer 1982). *D. antillarum* reach their maximum test size of 6-10 cm as they approach their estimated lifespan of 3-4 years (Ogden and Carpenter 1987).

Ogden and Carpenter (1987) observed that *D. antillarum* is sensitive to changes in water quality, and requires clean, oxygenated, circulating water without strong wave activity. *D. antillarum* has been documented thriving in areas with sedimentation and sewage input, likely due to the algal overabundance that occurs. However, *D. antillarum* is not common in areas where excess sedimentation and pollution are extremely heavy.

Mass-mortality and recovery

In January 1983, an unidentified waterborne pathogen decimated *D. antillarum* populations by an average of 95-99% across the entire Caribbean (Lessios 2016). The pathogen was first detected at Punta Galeta near the Atlantic Panama Canal entrance and spread throughout the Caribbean and western Atlantic over the course of one year (Lessios et al. 1984). The pathogen did not affect any other echinoids (Lessios et al. 1984). Although its origin is still unknown, the disease's spread via prevailing currents, its species-specificity, and laboratory data suggest that a waterborne pathogen caused the mortality (Lessios 2016).

Few areas in the Caribbean have consistent data on *D. antillarum* population densities, making it difficult to quantify the extent to which the pathogen afflicted populations (Lessios 2016). It is also challenging to compare population densities between sites because scientific methods and biases differ between censuses (Lessios 2016). Lessios (2016) analyzed *D. antillarum* density data from 20 different locations throughout the Caribbean, 14 of which had data on pre-mortality density. Where *D. antillarum* could once be observed in densities ranging from .76-14.38 individuals m^{-2} in these 14 locations, its average density was reduced to .01-.72 individuals m^{-2} immediately following the die-off.

It is difficult to consistently and accurately examine *D. antillarum* recovery across the entire Caribbean, but the general result of populations decreasing by 95-99% and now being at less than 1 individual m^{-2} is observable in most locations that have been monitored since the mass-mortality (Lessios 2016). Chiappone et al's (2013) study reports that in the Florida Keys, *D. antillarum* density before the 1983 mass-mortality was as high as 6 individuals m^{-2}, but in 2013 was lower than 1 individual m^{-2}. In St. John, pre-1983 densities were up to 14 individuals m^{-2}, but were wiped out to below 0.2 individuals m^{-2} after the mortality (Levitan 2014). Some areas in Puerto Rico had 13-18 individuals m^{-2} before the die-off, but in 2011 their densities were between 0-0.44 individuals m^{-2} (Ruiz-Ramos et al. 2011). Population densities have remained near 0 at Panamanian and Floridian reefs (Lessios 2016). Most recovery has been observed in shallow depths, but the largest recorded post-mortality densities at depths >5 m are .74-2.27 individuals m^{-2} in Banco Capiro, Honduras (Bodmer et al. 2015). Lessios (2016) concluded that in 2015, with each location's changes calculated separately, Caribbean *D. antillarum* populations were generally still 8.5 times less dense than they were before the die-off.

Lessios (2016) also found that, in 12 of the 14 locations for which there was pre-mortality data, populations were increasing at slow, but positive rates of <.0001 to .16 individuals m^{-2} yearly. Carpenter and Edmunds (2006) documented recovery in Belize, St. Croix, Barbados, Jamaica, Grenada, and Bonaire, and that higher urchin densities correlate with coral recruitment.

In Jamaica, Hughes (1994) reported a significant increase in mean test size of *D. antillarum* individuals since the die-off, with a significant decrease in the abundance of juvenile

urchins. Many Caribbean locations have experienced this same inverse relationship between test size and population density, and Levitan et al (2014) speculated that increased test sizes after the die-off occur as an adaptation to the lower population densities and the resulting increase in food availability. However, extremely low densities are still consistent, indicating that the larger body sizes are not adapting for improved fertilization success (Lessios 2016). In addition, not all populations have demonstrated size increases along with density decreases (Lessios 2016; Lessios 1984).

Lessios' (2016) conclusion for *D. antillarum* population recovery in the Caribbean is that in at least 19 locations, densities 18-32 years following the die-off ranged from 0.01 to 3.93 individuals m^{-2}. This data compilation demonstrates that *D. antillarum*'s recovery has been extremely slow and limited. This slow and limited recovery was unexpected, since *D. antillarum* produces about 1 million eggs per spawning, is extremely fertile, has planktonic larvae that can travel long distances, and *E. viridis* helps facilitate its recruitment (Lessios 2016; Lessios 1995). Randall et al (1964) found that *D. antillarum* can quickly recolonize low-density areas. In an experiment where *D. antillarum* was removed from 93 m^2 of reef, it returned to one-fourth of its original density in 48 hours due to its rapid ability to move into uncolonized territory. This data further demonstrates the confusion surrounding lack of adequate recovery from the pathogen.

There are many theories as to why *D. antillarum* population recovery has been extremely slow and, in some locations, nonexistent. Lessios (2016) narrowed these possibilities down to four main hypotheses. One hypothesis is that the pathogen is still persistent and is killing newly settled urchins. New symptoms of the disease have not been observed in over 30 years of study since the die-off, so some scientists believe it is unlikely that this hypothesis explains the pattern of limited recovery (Lessios 2016). However, Beck et al (2014) found that *D. antillarum* may have a compromised immune system compared to other Caribbean urchins. This could potentially explain why it was the only echinoderm affected by the 1983 pathogen, and perhaps why its recovery has been so inefficient.

The second theory is that not enough zygotes are being produced because the remaining *D. antillarum* individuals are not geographically close enough to promote successful fertilization. Lessios (2005) first described this possibility in reference to the Alee Effect, in which there are too few remaining individuals upstream to reproduce enough to overcome the species' normal mortality rates. This hypothesis is supported by the results of Levitan et al (2014) which found that *D. antillarum* does not reproduce at higher rates to account for lower numbers of individuals. Subsequently, the lower densities of *D. antillarum* make for lower fertilization success between individuals than when the urchins are present in their pre-mortality high densities. *D. antillarum*'s asynchronous breeding pattern also does not aid recovery, as only 5% of a population will breed at once (Bodmer et al. 2015). However, Feehan et al (2019) demonstrated that *D. antillarum* larval settlement success depends on specific oceanographic conditions such as passing eddies; larval influx occurs in the presence of these preferred events.

Bak (1985) and Miller et al (2009) both demonstrate that *D. antillarum* larvae recruit at significantly higher levels onto plastic plates that were less-heavily covered in filamentous algae. This provided the idea for the third hypothesis, that the macroalgal phase shifts following absence of *D. antillarum*'s grazing has prevented settlement of their juvenile urchins. Carpenter and Edmunds (2006) found significantly lower *D. antillarum* densities across their 6 sites in algae-dominated zones. This data is consistent with Bodmer et al (2015), wherein a lack of coral reef structural complexity prevented juvenile survival due to lack of shelter. This is yet another negative example of how phase shifts homogenize reef landscapes and reduce biodiversity.

The fourth main hypothesis for the slow recovery is that predators are exerting more pressure on the populations now that their preferred prey is less abundant (Lessios 2016). There is currently no evidence directly supporting an increase in predation on *D. antillarum*, however, Harborne et al (2009) found that predation by fish hinders urchin recovery in areas where these predators are abundant, such as marine reserves or areas where fishing is regulated. Most predation pressure likely occurs on very small and young individuals, making them even more difficult to find, and further complicating the study of recruitment success since the die-off (Lessios 2016; Levitan et al. 2014).

The explanation for *D. antillarum*'s poor recovery probably lies somewhere in between or in combination of these different hypotheses (Lessios 2016). Inconsistency and lack of reliably updated research on further recovery or lack thereof hinders the scientific knowledge base for current population demographics and recovery (Lessios 2016). The species' unexpected lack of recovery demonstrates the strong impact of this unique, large-scale disturbance (Lessios 1995).

Ecological roles

It was not until the pathogen decimated *D. antillarum* populations that scientists realized the extent of the species' ecological importance (Lessios 2016). In Caribbean coral reefs, *D. antillarum* is a keystone herbivore promoting coral reef health by grazing on algae (Bodmer et al. 2015). When present in high densities, *D. antillarum* consumes a reef's entire net daily primary productivity in the form of algae growing on corals (Bodmer et al. 2015). Dense *D. antillarum* populations in shallow (<6 m) water were found to be associated with reduced macroalgal cover (Carpenter and Edmunds 2006). When *D. antillarum* was experimentally removed from a reef for 5 days, herbivores removed 50% less algae, macroalgal biomass increased by 20%, and total ecosystem primary productivity decreased by 37% (Carpenter 1988). Carpenter and Edmunds (2006) found that, across 6 locations in the Caribbean, reefs with *D. antillarum* supported 10.3 times as many juvenile coral recruits as reefs without *D. antillarum*. In Jamaica, coral recruitment decreased by over 80% within 2 years of the *D. antillarum* die-off (Hughes et al. 2010). This finding that *D. antillarum* grazing supports coral recruitment is consistent with data showing that as *D. antillarum* densities increase, so does overall percentage of coral cover (Ruiz-Ramos et al. 2011). Coral reefs inhabited with dense *D. antillarum* zones show higher rates of juvenile coral recruitment, indicating *D. antillarum's* potentially important role in promoting coral reef recovery from macroalgal phase shifts (Idjadi et al. 2010). *D. antillarum* also provides shelter for many juvenile fishes as well as palaemonid shrimps that live between their spines (Randall et al. 1964).

When herbivorous fish are at low biomass due to impacts like fishing down the food chain, herbivorous urchins provide a trophic replacement by consuming enough algae to keep the coral healthy. (Graham et al. 2017). In an absence of herbivorous fish, grazing by *D. antillarum* alone is enough to reverse the macroalgal phase shifts plaguing many coral reefs (Mumby 2009). When *D. antillarum* are experimentally dispersed throughout a reef, therefore not exhibiting their usual grouping behavior, they compete less and consume more algae (Macia et al. 2007). These results suggest that *D. antillarum* population recovery could help reverse the phase shifts and reduced biodiversity plaguing many Caribbean reef systems (Macia et al. 2007; Mumby 2009).

Because of how important *D. antillarum* is in macroalgae regulation, the pathogen's reduction of their density in reef ecosystems directly caused and exacerbated macroalgal phase shifts throughout the Caribbean (Bodmer et al. 2015; Bak et al. 1984; Carpenter 1988). Increases in macroalgae cover diminish the energy availability of the reef and subsequently reduce

biodiversity (Bodmer et al. 2015). Hughes et al (2010) found less algal cover in the few locations *D. antillarum* has been able to repopulate. In a small minority of locations, grazing fish were plentiful enough to prevent phase shifts without *D. antillarum* present (Hughes et al. 2010).

Research Location

The isthmus of Panama is characterized by a wide variety of tropical ecosystems. The Guna Yala Comarca, on the Caribbean side, spans 480 km of Panama's northeastern coastline and contains 638 km^2 of coral reef around its 360 small islands (Andrefouet and Guzman 2005). The four main geomorphological categories of Guna Yala reefs are fringing reefs, deep reefs, coastal patch reefs, and reef complexes (Andrefouet and Guzman 2005). Western Guna Yala presents the most abundant and diverse reef formations in Panama (Andrefouet and Guzman 2005). Species of all five Echinodermata classes are present in Panama; 18 species in the urchin Class Echinoidea inhabit Panama's Caribbean coast (STRI 2008).

The Guna indigenous group inhabits primarily the islands of the Guna Yala Comarca and has been officially autonomously managing the area's marine resources since 1938 (Andrefouet and Guzman 2005). In addition to the global threats facing reefs, Guna Yala also has site-specific challenges with marine resource overexploitation and pollution (McEntee 2012). The size of the Guna population is increasing steadily and rapidly, so local people mine coral to add to the sizes of their islands (Guzmán et al. 2003). Coral mining decreased Guna Yala's coral cover by 79% from 1970 to 2001, while the indigenous population grew by 60% (Guzmán et al. 2003).

Fishing for *P. guttatus* and *P. argus* is a main income source for many families across the Guna Yala islands (Castillo and Lessios 2001). Moritz (2014) reports that local fishermen have noticed a lobster population decline in recent decades. Because it is such an important part of so many people's livelihoods, many fishermen still catch lobster below the minimum permitted size and are not always able to observe the restriction periods. Overfishing is also a major issue facing Guna Yala; surveys done by Colton (2011) demonstrate local fishermen awareness that fish populations have declined and that action needs to be taken to conserve this essential resource. Although many of the local indigenous people highly value the importance of marine resources for their communities, more education and awareness are needed in order to achieve widespread, sustainable compliance with fishing regulations (Nyquist 2014).

In addition to reef overexploitation by fishing and coral mining, Guna Yala's reefs are also endangered by the islands' pollution issues. According to a study by Fruitema (2015), as the Guna population increases, so does the demand for plastic, canned, and bottled goods. With no established sustainable waste management system in place, many families dump their waste on the nearest available shoreline or directly into the ocean. This results in contaminated waters and severe public health issues. The local community has expressed concerns regarding these public health issues resulting from unsustainable waste management practices, and some recycling initiatives are in practice. In surveys done by Nyquist (2014), interview participants local to three different Guna Yala islands almost unanimously agreed that trash is the community's largest environmental problem.

Diadema antillarum in Guna Yala

In the 17 Guna Yala reefs where *D. antillarum* has been studied, the initial die-off began in April 1983 and killed 98.9% of the population (Lessios et al. 1984). The next time data was reported for *D. antillarum* in Guna Yala was 10 years following the die-off, by Lessios (1995). The population densities had continued declining and were now on average <0 individuals m^{-2},

<3.5% of their pre-mortality levels. Average test size also declined by 10-20 mm, with a yearly average of 17.3-35.2 mm since 1984. The species remained absent in deep water. Lessios (2016) hypothesizes that average test size did not increase in San Blas like it did in other Caribbean locations because Guna Yala has, comparatively, lower fishing pressure than other Caribbean reefs. Lessios suggests that this has allowed fish to keep up with the algae cover, and therefore *D. antillarum* have not increased their algal consumption and grown larger.

Twenty years following the die-off, densities were at a maximum of 0.5 individuals m^{-2} during successful recruitment events (Lessios 2005). Panama is the only location in the Caribbean where *D. antillarum* does not exhibit a specific reproductive season, which could indicate that recruitment is relatively unsuccessful in Panama (H. A. Lessios, personal communication, November 14, 2019).

In this study, I monitored the status of *D. antillarum*'s population density in Guna Yala for the first time since 2015, as well as average test sizes for the first time since 1995. Similarities or differences between my density findings, previous densities, and other densities throughout the Caribbean help indicate the state of recovery in Guna Yala. Comparisons were made between the average test sizes I found, the average test sizes found at other Guna Yala reefs in the past, and at other Caribbean locations. I also investigated *D. antillarum*'s preferred substrate habitats, as well as the average test sizes of the urchins located on different substrate types. The substrate types that *D. antillarum* inhabit could indicate their preferred habitat niche within the reef, with potentially important implications for where members of this species can best recover and thrive, as well as the potential for phase shift prevention and reversal in Guna Yala.

The Smithsonian's Guna Yala research station closed and all research there was halted in 1998 when the Guna General Congress revoked permission for scientific study. It is important to continue monitoring *D. antillarum* populations in Guna Yala because it is one of the first locations the pathogen spread to, and therefore its conditions essentially reflect the persistence of the pathogen's effects on *D. antillarum*'s ecological communities. Test size has not been measured in Guna Yala in 24 years, and it has never been measured outside of the Cayos Limones. By making temporal comparisons between data from before and after the die-off throughout the Caribbean, my paper contributes to avoiding the 'shifting baselines' phenomenon by compiling data about the ways *D. antillarum* used to populate the Caribbean so that its unhealthy post mass-mortality status does not become the new norm. Establishing a current baseline for density, test diameter, and daytime substrate preferences at reefs west of Cayos Limones could aid in future comparisons as the recovery and population health of this species continues to be monitored over time.

Research Question
What are the population demographics of the *Diadema antillarum* among three shallow Guna Yala reefs and how do these data compare to other studies done on their recovery since the die-off?

Research Objectives
- Assess *Diadema antillarum* recovery 36 years after the 1983-84 die-off in three different Guna Yala shallow reefs by analyzing test size, population density, and substrate preferences

- Compare my data with the recovery in Guna Yala over time, as well as with other locations throughout the Caribbean

Methods

Study Area

This study was conducted off of Isla Wichub Wala in Panama's Guna Yala Comarca, at three reef sites: Ukuptupu, Aguadargana, and Pico Feo (Figure 1). Temperature data was obtained from AccuWeather and ranged from 23-33º C during the study period. Sea surface temperature data was acquired from Sea Temperature Info and averaged 29º C during the study period, which is the warmest sea water in Panama at the time of year when this study was conducted.

Ukuptupu reef (9º33'07.06"N, 78º57'11.46"W) is located approximately 30 m offshore of the southeastern side of Isla Ukuptupu (Sand Island), which is about 50 m west of Wichub Wala. Ukuptupu and Wichub Wala are both inhabited islands. This is an extremely shallow reef, and investigations were conducted at depths ranging from 0.25 m to 1 m. When it was visited for this study, the Ukuptupu reef was dominated by high concentrations of trash, dead coral, fire coral (*Millepora alcicornis*), finger coral (*Porites porites*), algae, sea sponges, and warm, oily water. Ukuptupu is the local name for what was described in Lessios (1984) as "House Reef" because it is right outside where the inactive Smithsonian Research Center is located (H. A. Lessios, personal communication, November 14, 2019).

Aguadargana reef (9º33'01.51"N, 78º56'53.63"W) is about 100 m offshore of an inhabited island and is a site with extremely high evidence of coral mining. Measurements were taken at depths ranging from 1-3 m. Similar to Ukuptupu, there was a lot of trash and warm, oily water present. Sections of the reef were very obviously dead and mined, while other sections appeared healthy. Most coral skeletons were dominated by algae; algae had a very large presence at this site.

Pico Feo reef (9º33'11.15"N, 78º58'11.05"W) is located about 70 m offshore of an uninhabited island. There was visual evidence of coral mining on the reef's outskirts, but the site was mostly characterized by patches of healthy reef, sandy substrate, large amounts of *Porites porites*, and dead coral covered in algae. Measurements were taken at depths of 1-2 m. The water at Pico Feo was hardly turbid and generally appeared quite clear. Pico Feo has never been studied for *D. antillarum* density before because of its brackish water quality due to runoff from a nearby stream (H. A. Lessios, personal communication, November 14, 2019).

Figure 1. Photos of study location acquired from Google Maps and Google Earth Pro. Local names were used. (A) Regional location of study (near El Porvenir) in relation to the country of Panama and its Guna Yala Comarca (B) Study sites (indicated by stars) located off the coasts of various islands in proximity to Isla El Porvenir.

Data Collection

      Data were collected by snorkeling during Panama's rainy season, from November 7-12, 2019, in mid-morning to early afternoon. Data collection occurred before the day's first high tide. Tidal information was obtained from Tides Chart (tideschart.com); tides averaged at about .35 m during data collection times. Data were collected during *D. antillarum*'s prime reproductive season in the rest of the Caribbean excluding Panama (H. A. Lessios, personal communication, November 14, 2019).

At each site, three 30 m belt transects parallel to shore were surveyed 2 m to the right and left of the transect tape (Blanco et al. 2010). 360 m^2 of reef were surveyed per site. Each transect's starting location was based on swimming in a random direction for about 20-30 seconds and starting the transect tape at that point. A visual survey within the 2 m range to the left and right of the transect tape was conducted by slowly snorkeling along the transect line and diving to carefully inspect every three-dimensional element and crevice of the reef within the transect area (Idjadi et al. 2010). The 2 m distance to the left and right of the transect line was gauged using a 2 m long PVC pipe.

Each *D. antillarum* individual was counted, its location along the transect recorded, its location on the substrate described, and, when possible, measurements were taken to the nearest millimeter using calipers (Ritz 2008; Chiappone et al. 2013). The diameter of urchins' peristomial plating across the mouth on its underside was measured at Ukuptupu and Aguadargana. Both the peristomial mouth diameter and the full test diameter were measured at Pico Feo. Visual surveillance was conducted of each site's and transect's general habitat structure and substrate composition (Ritz 2008).

Data Analysis

A regression relating Pico Feo's two types of urchin measurements was performed as a way to work with a different type of collected data that can be normalized to test size. The regression equation was used to calculate the test diameters for Ukuptupu and Aguadargana's urchins based on the peristomial mouth diameter measurements. Regression data is presented separately in the Appendix.

Density of *D. antillarum* was calculated for each reef. One-way ANOVA analysis was performed to determine if there were any statistically significant differences between the sites' mean densities (Blanco et al. 2010). All statistical analyses were done using MS Excel. To calculate the overall density, the mean densities of each of the three sites were averaged together and then divided by the total 1,080 m^2 surveyed.

One-way ANOVA was performed to see if the urchins' average test sizes differed significantly between sites (Blanco et al. 2010). Each site's urchins were categorized into size classes in increments of 0.5 cm from 0 to 7 cm (Lessios et al. 1984; Levitan et al. 2014). A histogram was created for each site and overall showing the number of urchins in each size class. Juveniles were identified as having test sizes <2 cm, middle-aged urchins were identified as ranging from 2-6 cm, and older urchins were identified as nearing the species' maximum test sizes from 6-10 cm (Lessios 2016; Randall et al. 1964; H. A. Lessios, personal communication, November 14, 2019; Ogden and Carpenter 1987).

Four substrate types were identified during the sampling. The number of urchins in each substrate category was determined for each site. Mean test sizes for the individuals in each substrate category were calculated for each site. One-way ANOVA was performed to see if there was a significant difference between the average test sizes of urchins located on the different substrate types at each site.

Ethical considerations

The Local Review Board (LRB) and the SIT Institutional Review Board (IRB) both approved this project. No human subjects were involved in this research, but precautionary measures were taken to minimize the study's impact on the environment and local community. I did my best to conduct myself in a respectful and gracious manner during the entirety of my time

in the Guna Yala community. I stayed far out of fishermen's way if I saw them while conducting research so as not to disturb them while they worked. I used reef-safe sunscreen every time I went into the field and made sure to use my equipment for the bare minimum amount of time necessary as to reduce my study's impact on the reefs. We transported to shared research sites in groups so as to minimize boat traffic and emissions. No equipment was ever left in the field between or after research visits. When laying transects, I avoided my tape touching or wrapping around anything within the reef to the best of my ability. I placed my rebar carefully in sandy patches or holes in the coral so that the coral remained as it was before I arrived. I handled every urchin with extreme care by trying not to damage its fragile spines and holding it in a position where it was not likely to be damaged if a current moved my hand that was holding it. I placed each urchin back exactly where I found it after taking my measurement. Finally, I never touched anything I saw in the reef besides the urchins I was measuring, and I only ever stood up on sandy substrate so as not to damage any living elements of the reef. All trash that was generated during the research period on Wichub Wala Island was carried off of the island upon project conclusion and was disposed of or recycled in Panama City.

Results

A total of 119 *Diadema antillarum* were found in the total 1,080 m^2 reef area surveyed (0.11 individuals m^{-2}). *D. antillarum* densities were 0.08 individuals m^{-2} (n=28) at Aguadargana, 0.12 individuals per m^{-2} (n=44) at Ukuptupu, and 0.13 individuals m^{-2} (n=47) at Pico Feo (Figure 2). There were no significant differences (p<0.05) between the three sites' urchin densities (Figure 2).

Figure 2. Mean *Diadema antillarum* densities m^{-2} at Ukuptupu, Aguadargana, and Pico Feo reefs. Error bars are standard deviation from the mean of the three transects conducted at each site. One-way ANOVA p>0.05.

The average test size of the 60 total urchins measured was 38.8 mm. *D. antillarum* average test sizes were 31.1 mm at Ukuptupu (n=16 urchins measured), 39.8 mm at Aguadargana (n=18 urchins measured), and 45.6 mm at Pico Feo (n=25 urchins measured)

(Figure 3). The statistical one-way ANOVA test did not find a significant difference (p<0.05) between the three sites' mean test sizes (Figure 3).
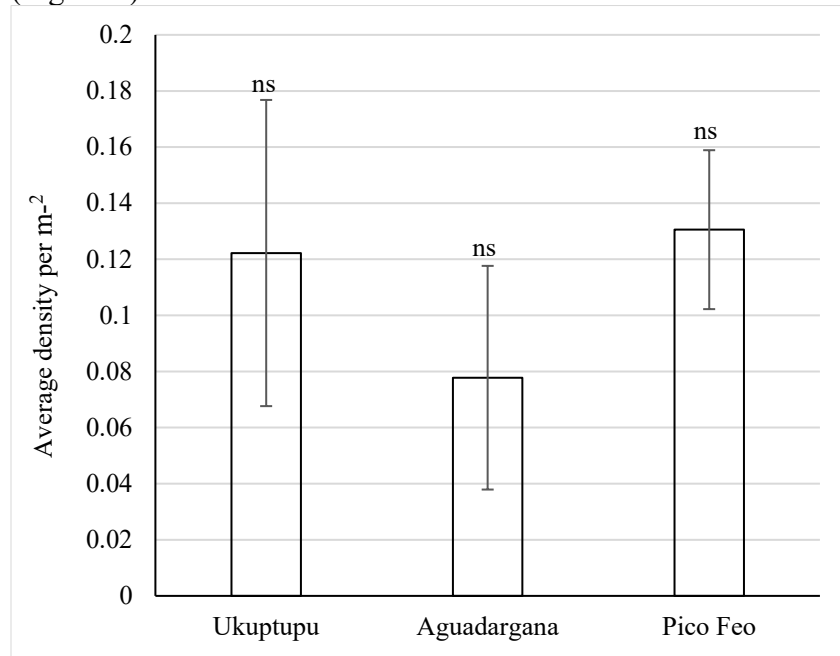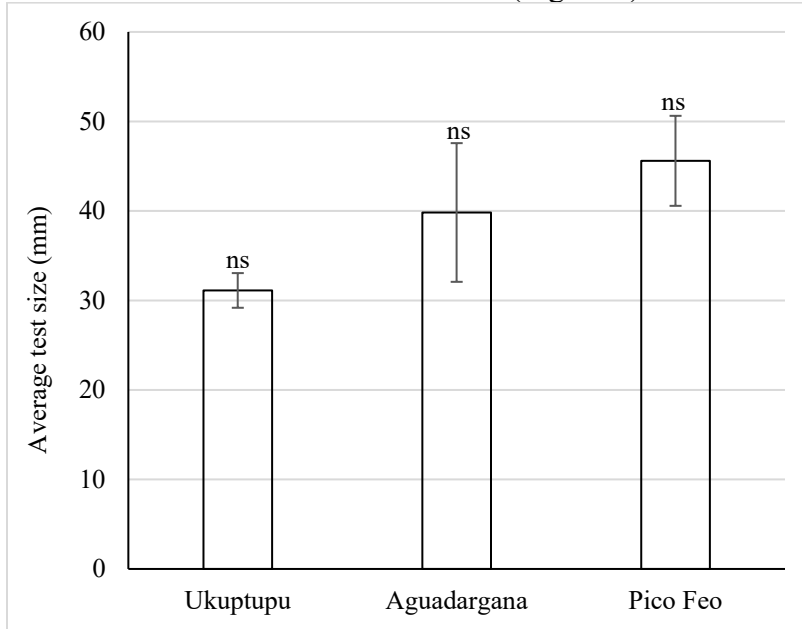

Figure 3. Mean *Diadema antillarum* test sizes at Ukuptupu, Aguadargana, and Pico Feo sites. Error bars represent standard deviation from the mean of the three transects conducted at each site. One-way ANOVA p>0.05.

       Ukuptupu's urchins were all mid-life stage, with test diameters between 2 and 4.5 cm, and the majority around the overall mean of 3.8 cm (Figure 4A).

       Aguadargana's urchins exhibited a wider range of test sizes, between 1 and 6.5 cm (Figure 4B). Two juveniles (test size<2 cm) were found at Aguadargana along with one 62 cm urchin in *D. antillarum*'s maximum test size range between 6 and 10 cm (Figure 4B). The majority of Aguadargana's urchins were also middle-aged, between 4 and 5 cm (Figure 4B).

       Pico Feo exhibited the largest range of test sizes, from .5 to 6.5 cm (Figure 4C). This site had 5 individuals with test diameters >6 cm, and 3 juveniles (Figure 4C). The majority of Pico Feo's urchins still fell in the middle-aged zone, between 4 and 5.5 cm (Figure 4C).

       Although the mean test size between all three reefs was 3.8 cm, Figure 4D demonstrates that only 3 urchins fell in the range of 3.5-4 centimeters, while the majority measured greater than 4 cm. Most individuals were mid-life stage (H. A. Lessios, personal communication, November 14, 2019).
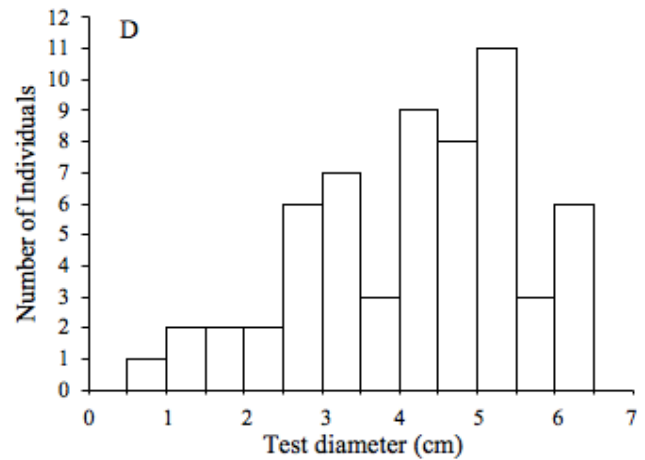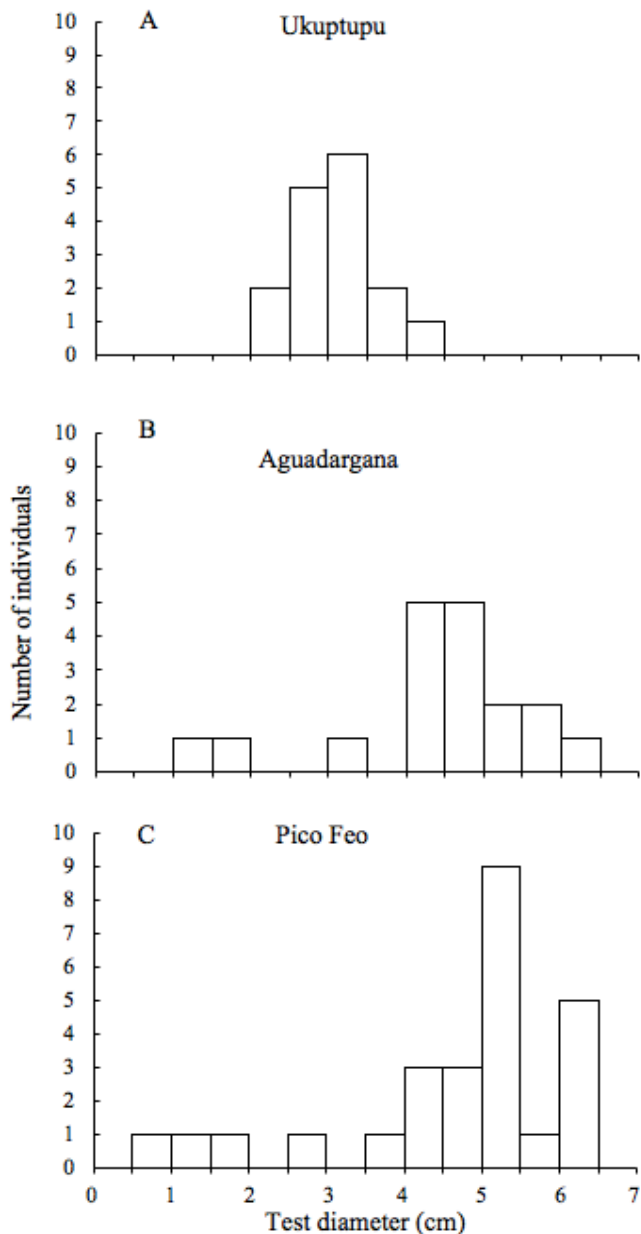
Figure 4. Histograms representing the total number of *D. antillarum* individuals in each size category for (A) Ukuptupu reef, (B) Aguadargana reef, (C) Pico Feo reef, and (D) all individuals from all reefs combined.

At all three reefs, urchins tended to aggregate and densely inhabit areas with more diverse topography and hiding places. *D. antillarum* were observed living in groups of 2-8 individuals, but it was not uncommon to encounter solitary *D. antillarum* as well. Every site had an extremely prolific presence of *E. viridis* and rock-boring urchins (*Echinometra lucunter*), even on flatter areas of the reef without geographic complexity.

Urchins were encountered in 4 different categories of substrate. "Within live coral patch" refers to urchins that were visible in coral craters and crevices from a vantage point of directly above them. "Under live coral" refers to urchins that were found hiding under coral in crevices that made them impossible to see without diving down close to the coral and inspecting it from every angle. "Under dead coral" refers to the dead coral structures covered in algae that had a strong presence at every site and provided many different types of crevices in which urchins hid.

"Under rock" refers to urchins that were found hiding underneath rock structures. It was not possible to measure all urchins at each site, so the n-values in Figures 5A, 6A, and 7A do not appear to match those in Figures 5B, 6B, and 7B.

At Ukuptupu, 31 individuals were found under dead coral, 7 were found under live coral, 4 were found within live coral, and 2 were found under rocks (Figure 5A). Urchin test sizes in each category were pretty evenly distributed at this site (n=16 measured) (Figure 5B). The statistical one-way ANOVA test found no significant ($p < 0.05$) differences in mean test sizes between the substrate types.

A   B

Figure 5. Ukuptupu (A) total number of urchins found on each substrate type, (B) the average size of the urchins found in each substrate category; n= number of urchins measured; error bars represent standard deviation from the mean test size within each substrate category. One-way ANOVA $p > 0.05$.

At Aguadargana, 2 urchins were found at 2.5 m depth within the same live brain coral (*Diploria labyrinthiformis*) patch and 26 were found under dead coral (Figure 6A). Only one of the 2 found on live coral was able to be measured, so it is difficult to make comparisons between test sizes at this site (Figure 6B), and the results of the statistical one-way ANOVA test yielded no significant ($p < 0.05$) differences in mean test sizes between the substrate types.

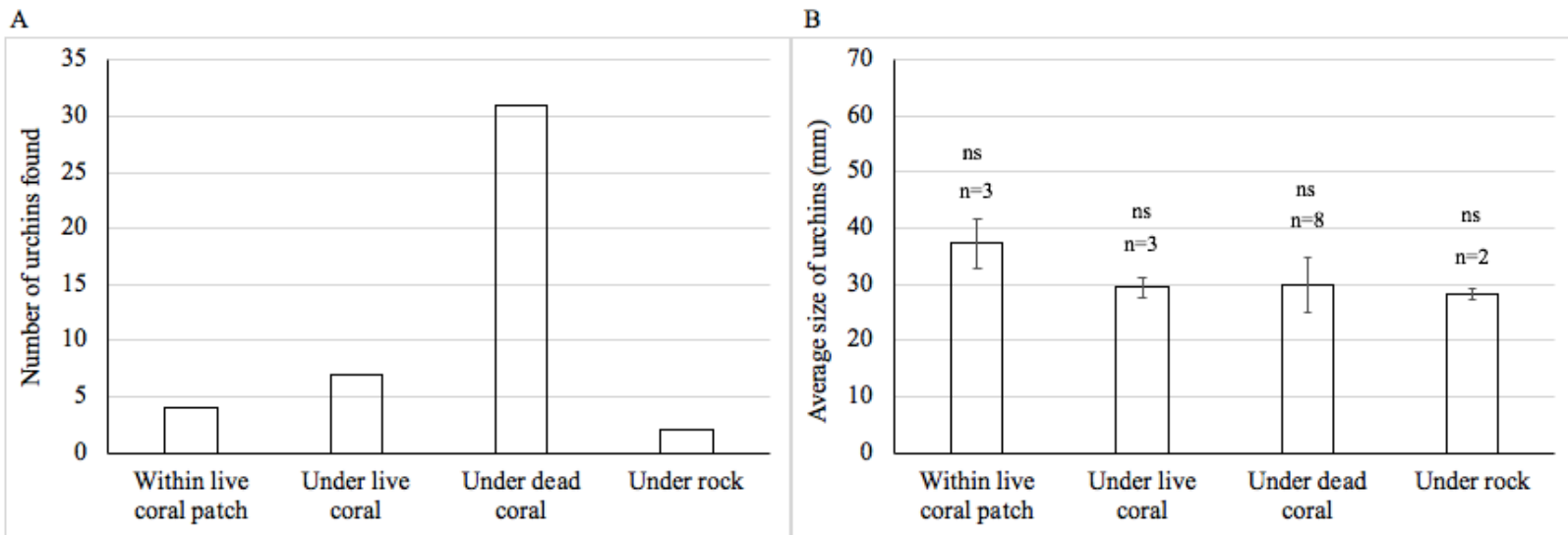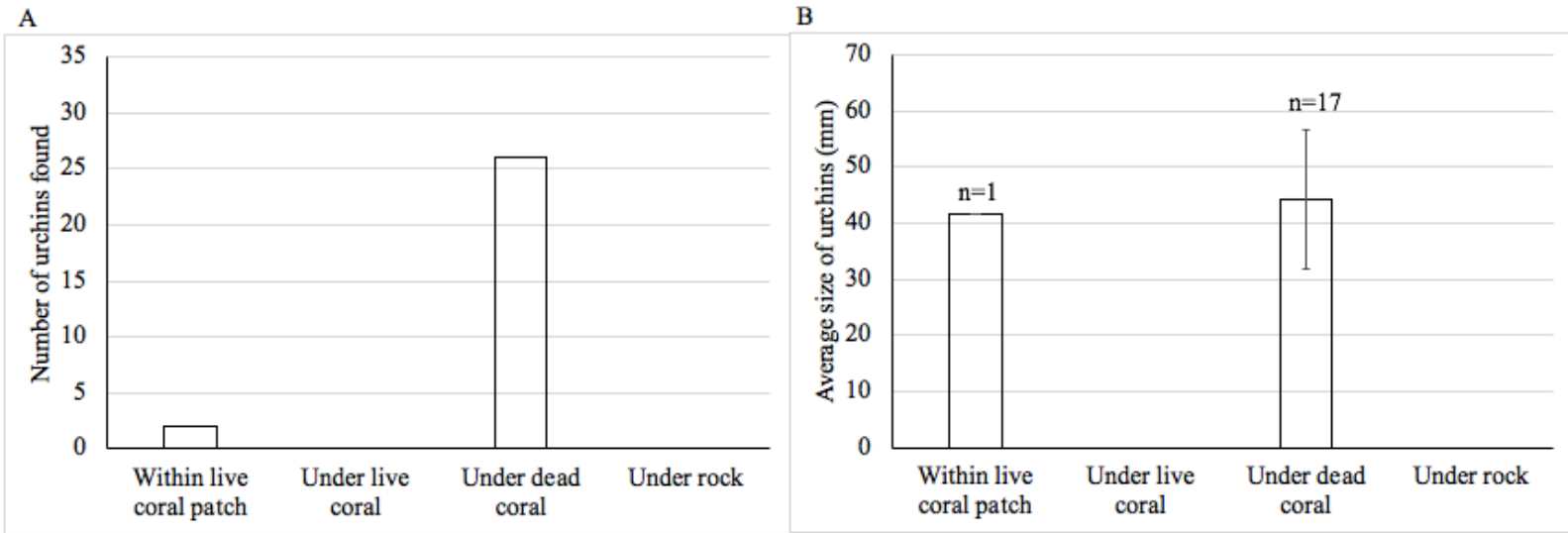**Figure 6.** Aguadargana (A) total number of urchins found on each type of substrate category, and (B) the average size of the urchins found in each substrate category; n= number of urchins measured; error bars represent standard deviation from the mean test size within each substrate category. One-way ANOVA p>0.05.

At Pico Feo, 37 individuals were found under dead coral, 4 were found under live coral, and 3 were found both within live coral and under rocks (Figure 7A). Urchin test sizes in each category were pretty evenly distributed at this site (n=25 measured), with no significant (p<0.05) differences in the mean test sizes per substrate type found by the one-way ANOVA (Figure 7B). Out of the three sites, the greatest number of urchins was able to be sized at Pico Feo.
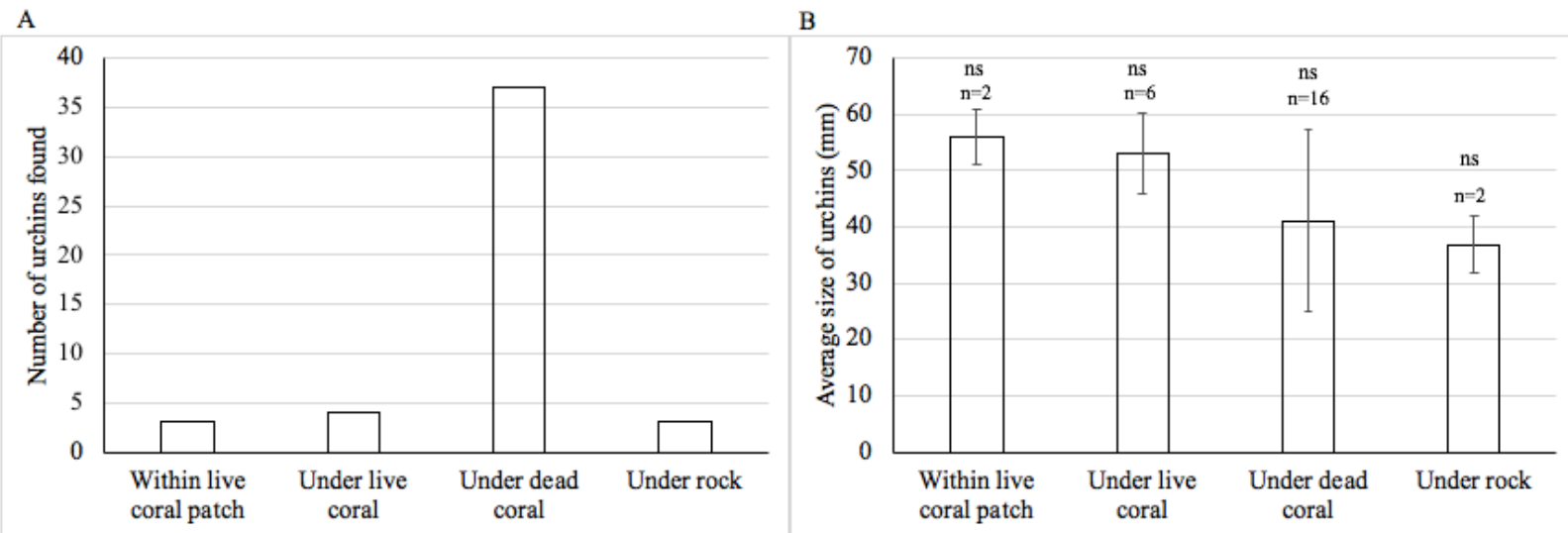
Figure 7. Pico Feo (A) total number of urchins found on each type of substrate category, and (B) the average size of the urchins found in each substrate category; n= number of urchins measured; error bars represent standard deviation from the mean test size within each substrate category. One-way ANOVA p>0.05.

Combining data from all three reefs, 94 urchins were found under dead coral, 11 were found under live coral, 9 were found within live coral, and 5 were found under rocks. *D. antillarum* exhibited a strong preference to be under the dead, algae-covered coral at every reef.

The 9 urchins found within live coral were the only urchins visible from directly above; the rest were underneath various structures and were found by more thorough searching. Of the urchins found within live coral, all but the 2 within *D. labyrinthiformis* at Aguadargana were found within *P. porites*. Urchins were found under two types of live coral; 2 were found under massive starlet coral (*Siderastrea siderea*) at Ukuptupu and 3 were found under *P. porites* at Pico Feo.

The mean test size between all sites for within live coral is 44.3 mm, 45.2 mm under live coral, 40.2 mm under dead coral, and 41.2 under rock. Evidently mean test sizes were similar among substrate types, and all of the values had similar standard deviations from the mean, between 9-14 mm.

Discussion
Density

The fact that the one-way ANOVA between the three sites' densities did not find any significant differences demonstrates that the densities at all three sites were uniform and consistent. Each site is unique and likely has its own factors acting on *D. antillarum* populations, but the consistent density results suggests that none of the sites have any factors affecting *D. antillarum* densities in a significantly unique way compared to the other sites. This bodes well for this study's data being representative of *D. antillarum* densities in western Guna Yala reefs.

The mean density of 0.11 individuals m^{-2} across all three sites is only 3.03% of Guna Yala's last recorded pre-mortality density of 3.63 individuals m^{-2} in 1982. In 1983, immediately following the mortality, density in the 11 reefs studied had fallen to .24 individuals m^{-2} (Lessios et al 1984). In 2015, the mean density was 0.03 individuals m^{-2} (Lessios 2016). Densities at all of the 11 Guna Yala reefs studied for density showed evidence of continued decline since the die-off, with a calculated average population growth rate of -0.66 individuals m^{-2} per year (Lessios 2016). If my data are representative of the study area, my 2019 density of 0.11 could indicate that the populations have started to have a positive population growth rate and could be recovering from their lower 2015 densities. Further study of more reefs in the area could help confirm this potentially hopeful result. Interestingly, the mean density found in this study was very similar to the 0.12 individuals m^{-2} found in Bocas del Toro, Panama 25 years after the mortality (Ritz 2008).

In 1982, before the mortality, 282 m^2 surveyed at Aguadargana yielded a mean density of 1.3 individuals m^{-2}, and the post-mortality 472 m^2 surveyed in 1983 yielded a 0.07 mean density (Lessios et al. 1984). In this 2019 study, 360 m^2 surveyed at Aguadargana yielded a mean density of 0.08 individuals m^{-2}, a slight improvement from the 0.07 mean density immediately following the die-off. However, slightly less area was surveyed in my study which could affect this comparison.

In 1982, before the mortality, 94 m^2 surveyed at Ukuptupu yielded a high mean density of 10.98 individuals m^{-2}, and the post-mortality 108 m^2 surveyed in 1983 yielded a 0.43 mean density (Lessios et al. 1984). My study's 360 m^2 survey at Ukuptupu yielded a mean density of 0.12 individuals m^{-2}, a drop from the density immediately following the die-off.

It is important to note that the environments at these locations have likely changed since the 1982 and 1983 studies were done. The increase in Guna Yala's population size likely caused more coral mining and pollution to impact these reef sites (Guzmán et al. 2003), which could also be contributing to *D. antillarum*'s recovery curve in these locations-- especially at Ukuptupu which is especially close to inhabited islands and could be more polluted than in the past.

Ogden and Carpenter's (1987) finding that *D. antillarum* can survive well in areas with high sedimentation and sewage levels could explain why the density at Ukuptupu was similar to Pico Feo's even though Pico Feo is a much cleaner site further from inhabited islands. The fact that Pico Feo's and Ukuptupu's densities are comparable suggests that, although Ukuptupu's pollution levels are visibly elevated and present, they are not high enough to prevent *D. antillarum* surviving to the same extent that they do in the cleaner waters of Pico Feo.

Pico Feo had a higher average density and a higher number of *D. antillarum* individuals than Aguadargana and Ukuptupu where studies have been conducted in the past. This result suggests that *D. antillarum* are able to survive in water with brackish quality, however, I did not conduct water quality measurements, so those would need to be done in order to confirm this possibility.

The observation of high amounts of *E. viridis* and *E. lucunter* supports the possibility that these urchin species could be replacing *D. antillarum*'s herbivorous role in reefs as suggested by Lessios et al (1984). Lessios' (1984) study compared *D. antillarum* population demographics in Panama before and after the die-off, thus noting many of the disease's symptoms as they manifested. The first visually evident symptom of the disease was sediment accumulation on the spines and flaking off of the outer skin. At Ukuptupu, three of the *D. antillarum* that I counted had light, brown gunk sitting on some of their spines, which could suggest persistence of the disease, but further research and testing needs to be done to investigate this observation.

The increased predation hypothesis that Lessios (2016) presents could possibly be less of an issue in Guna Yala due to the overfishing of *P. argus* by local communities (Moritz 2014; Castillo and Lessios 2001). Lower abundance of this predator could be contributing to population recovery in the area, although recovery is still very slow as documented by my study in comparison to the 2015 and earlier densities.

Test size

The fact that the one-way ANOVA between the three sites' test sizes did not find any significant differences demonstrates that the test sizes at all three sites were relatively uniform and consistent. Like with the density data, this result suggests that the three reefs are experiencing similar patterns in terms of *D. antillarum* population status and recruitment levels.

Before the die-off, average test size in the Cayos Limones was 42.2 mm. After the die-off, average yearly sizes were significantly lower, ranging from 17.3-35.2 mm from 1984-1995. This 2019 study's mean test size of 38.85 mm is still relatively small, especially given that Carpenter and Edmunds (2006) characterized "juveniles" as all *D. antillarum* with test sizes <30 mm. However, my 38.85 mm average approaches the pre-mortality average of 42.2 mm and Figure 4D demonstrates that 37 out of the 60 measured individuals were actually well-above the average size; the tiny juveniles brought the average down.

Slightly increased average sizes for *D. antillarum* in Guna Yala could mean several things. It could mean that survivability is higher than it was right after the die-off, and individuals are living longer and thus achieving larger test sizes. Larger test sizes could also indicate greater food availability due to macroalgal phase shifts, which is what was observed in many other Caribbean habitats after the die-off (Hughes 1994; Chiappone et al 2013; Martín Blanco et al 2010; Levitan et al 2014). However, an increase in food abundance is unlikely to be the case for Guna Yala's urchins because the average test size found in my study did not exceed the pre-mortality average. This study's three reefs do not have large average test sizes compared to other Caribbean locations where size has significantly increased since the die-off. Chiappone

et al (2013) reported that mean *D. antillarum* test sizes in the Florida Keys significantly increased with decreased densities since monitoring began in 1999. The test sizes found in this study ranged from 2.1-5.9 cm. In Cuban reefs, Martín Blanco et al (2010) also found an inverse correlation between average test size and population density, with an average test size of 5.7 cm. In St. John, Levitan et al (2014) also found a significant increase in test sizes with decreased densities since the mass-mortality. The study found that, in 2011, average test size was 4.5-8 cm, a significant increase from the 2.5-5.5 cm average in 1983. In Bocas del Toro, Panama 25 years after the mass-mortality, the average test size was 67.8 mm, much larger than the Guna Yala average found in this study (Ritz 2008).

Five juveniles (test size<2 cm) were found out of the 60 total urchins measured, and one additional juvenile was noted outside of a transect at Pico Feo. This low density of juveniles compared to adults is consistent with the findings of Levitan et al (2014) and Carpenter and Edmunds (2006), and is also consistent with the fact that juveniles are extremely cryptic and vulnerable to predation (Lessios 2016). With the mass-mortality causing a reduced density of adults, it is increasingly challenging to find surviving juveniles due to lower levels of recruitment capability (Lessios 2016). However, most of the individuals measured were between 3-6 cm, which is when *D. antillarum* are sexually mature, which could indicate current higher reproductive potential.

Carpenter and Edmunds (2006) found that higher density of juveniles indicated higher recruitment levels in those areas. Based on my results, it appears that Aguadargana and Pico Feo may have higher levels of recruitment than Ukuptupu, where no juveniles were found. However, the total of only 5 juveniles likely still indicates a low level of recruitment in these reefs (H. A. Lessios, personal communication, November 14, 2019).

Ukuptupu had the smallest range of test sizes and no juveniles or older (test size>60-100 mm) individuals were encountered (Figure 4). All were middle-aged, sexually mature adults. The apparent lack of juvenile recruitment and longer lifespans could be a result of the site's high pollution levels and proximity to inhabited islands (Ogden and Carpenter 1987). Aguadargana had approximately 15 fewer urchins than Ukuptupu, yet still had a wider range of test sizes possibly because of the distance from direct pollution from shore, and because of the large algae-dominated coral presence.

Substrate

The fact that the overwhelming majority of *D. antillarum* , 94 out of the total 119, were encountered under dead coral structures covered in algae is logical given *D. antillarum*'s algae diet and its cryptic daytime behavior (Randall et al. 1964). Since these structures were made of dead coral skeletons and not live coral with polyps, there were more holes and crevices that urchins were able to access and hide in; this is an easier place to live and maneuver than, say, within the dense terrain of live *P. porites*.

The fact that the large majority of urchins were located on dead coral skeletons covered in algae supports the research on *D. antillarum*'s potential ability to reverse macroalgal phase shifts (Hughes et al. 2010; Mumby 2009; Carpenter and Edmunds 2006). It is promising to see that the few *D. antillarum* that are present in these Guna Yala reefs are performing their extremely important role as herbivorous inhabitants of algae-dominated areas. However, due to their continued low densities found in this study, it is unlikely that there are large enough *D. antillarum* populations in these three Guna Yala reefs for the individuals to be significantly

promoting coral recruitment and reducing algal cover the way they are able to when present in high abundances (Carpenter and Edmunds 2006).

The Aguadargana reef had the fewest urchins out of the three sites, and all but two of these individuals were located on dead coral structures. This site had the heaviest coral mining evidence of the three sites, so the only substrate left in *D. antillarum*'s preferred shallow waters may be dead coral, which is usually algae-dominated (Mumby 2009). Aguadargana was also a generally deeper site than the other two, which could also explain its lower urchin density.

It is possible that the large presence of algae-covered coral skeletons could be inhibiting juvenile *D. antillarum* recruitment, as suggested by Bak (1985) and Miller et al (2009)'s data showing that juveniles recruited better in areas with less algae dominance. Bodmer (2015) also found that reefs with high algae-dominance possessed less habitat heterogeneity and subsequently less juvenile survivability due to the lack of shelter. However, all 5 of the juveniles found in this study were located on algae-covered dead coral, which suggests that the high presence of algae is not completely preventing *D. antillarum* juvenile survival and recruitment in these reefs.

Aside from the fact that all 5 juveniles were found on the algae-covered coral skeletons, the rest of the mean test sizes were very evenly distributed among the different substrate types. The overwhelming majority of *D. antillarum* preferred to live under dead coral structures covered in algae, no matter what test size they were. The fact that there was no significant difference between where *D. antillarum* of varying test sizes preferred to live suggests that individuals of varying sizes are all able to live in different substrates. This result indicates that something besides test size dictates the substrate type urchins choose to inhabit. Further research could be done to investigate this specific phenomenon.

Possible error sources and limitations

It is important to note that previous density studies done in Guna Yala surveyed 11 different reefs and at depths of 1-17 m, or until the reef ended, so the differing scope and methods make it difficult to compare between censuses (Lessios et al. 1984; Lessios 1995; Lessios 2005; Lessios 2016). After the die-off, *D. antillarum* was absent from deeper areas (Lessios 1995), but my study did not investigate its presence in depths >3 m due to the limitations of snorkeling. Due to environmental conditions like currents and cryptic daytime behavior, different percentages of the total number of urchins encountered at each site were actually able to be measured. I tried to measure as many urchins as possible, but I also did not want to damage them by trying too hard to dig them out of their hiding places. Varying weather conditions at different sites on different days made it so that more urchins were able to be measured at some sites than others. Having more consistency between percent of the total number of urchins measured at each site could have helped the data be more representative. The regression analysis and quantification of the relationship between two different types of measurements that can be taken on sea urchins was important to do. However, it would have been useful to measure the test sizes of every individual at Aguadargana and Ukuptupu in order to have more data for a more precise regression. With this study's temporal and geographic limitations, I prioritized gathering data from reefs where test size had never been studied before instead of studying in the Cayos Limones where test size had been previously measured. Since test size was measured at novel sites, it was assumed that these sites had similar impact from the pathogen as did the previously-surveyed Guna Yala reefs.

Conclusion

   Diadema antillarum's mean density in this study was only 3.03% of the 1982 pre-mortality density, but still higher than the 2015 density, potentially indicating recovery rather than population decline in the last 5 years. My study's mean density at Aguadargana demonstrated slight improvement from the 1983 survey immediately following the die-off. My study's mean density at Ukuptupu showed a prominent decrease from the 1983 density immediately following the die-off, which could be due to pollution levels. Pico Feo had never been studied for *D. antillarum* density before because of its brackish water quality, however, my study demonstrated that *D. antillarum* persist there.

   The overall mean test size was slightly higher than the average for the 10 years following the die-off, but still below the pre-mortality average. Five juveniles were encountered between Aguadargana and Pico Feo, suggesting that recruitment is still occurring at these sites, albeit at low levels (H. A. Lessios, personal communication, November 14, 2019). All juveniles were found on the same type of substrate, algae-covered dead coral skeletons, supporting that this species can recruit in reefs with high levels of algae. The large majority of urchins at every site were located on dead coral structures covered in algae. The second most popular substrate type was under live coral at 11 total individuals, then within live coral at 9 total individuals, followed by under rocks at 5 total individuals. There were no significant relationships between urchin test sizes and the type of substrate they resided on, suggesting that something other than test size determines where *D. antillarum* choose to be located within a reef, and that *D. antillarum* in Guna Yala could have the potential to aid in phase shift reduction due to their exhibited preference for algae-covered surfaces.

   Future studies testing factors that influence *D. antillarum* substrate preferences within reefs could provide insight into what kind of reef environments best lend to the species' recovery. Returning to Ukuptupu and conducting tests to figure out why some urchins have brown accumulations on their spines could be very helpful in confirming or eliminating the possibility that the disease is persisting in this area. To expand on my study, more transects should be conducted in at least 8 more reefs in Guna Yala, preferably with the use of SCUBA equipment to investigate greater depths. Water quality measurements testing the salt content of the water at Pico Feo should be conducted to investigate the current status of the potentially brackish content. The insignificant differences between average densities, test sizes, and substrate preferences between the three sites suggest that the reefs are experiencing similar population recovery phenomenon and could be representative of *D. antillarum* status in Guna Yala coral reefs.

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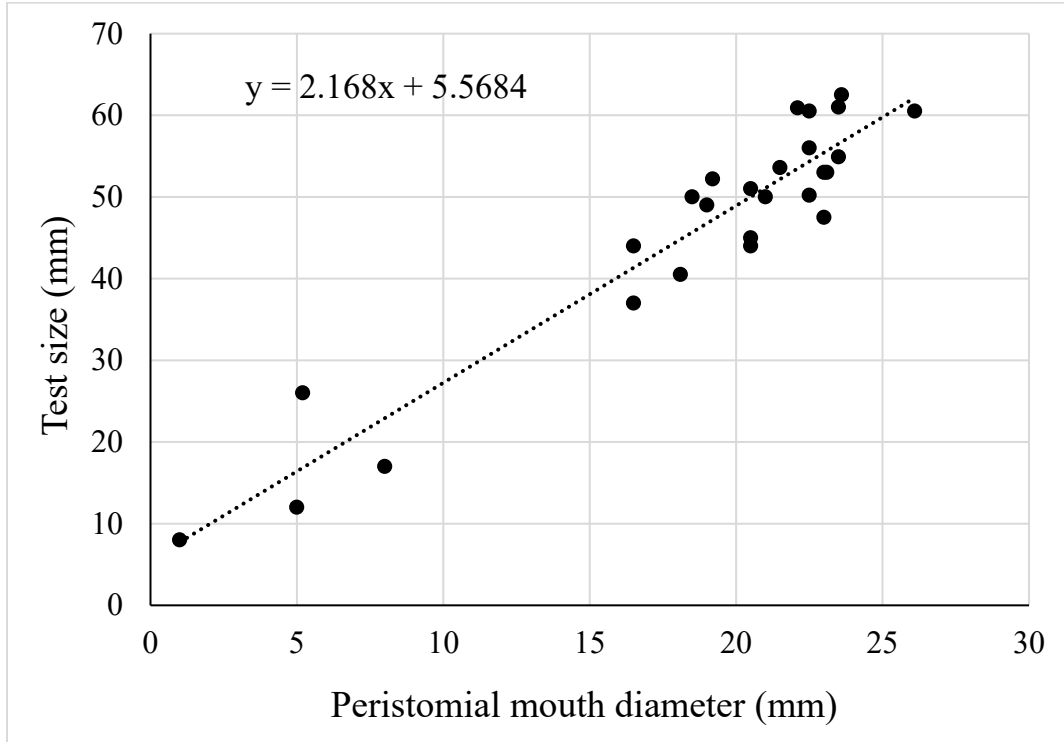
Regression of Peristomial mouth diameter (mm) with total test diameter (mm) measurements at Pico Feo