

Current status of the sea
urchin *Diadema antillarum*
in Puerto Rico

by

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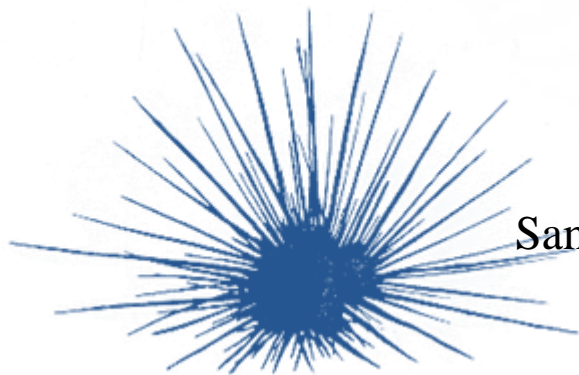
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CURRENT STATUS OF THE LONG-SPINED SEA URCHIN

DLADEMA ANTILLARUM IN PUERTO RICO

To my family, friends, enemies, and *Diadema antillarum*

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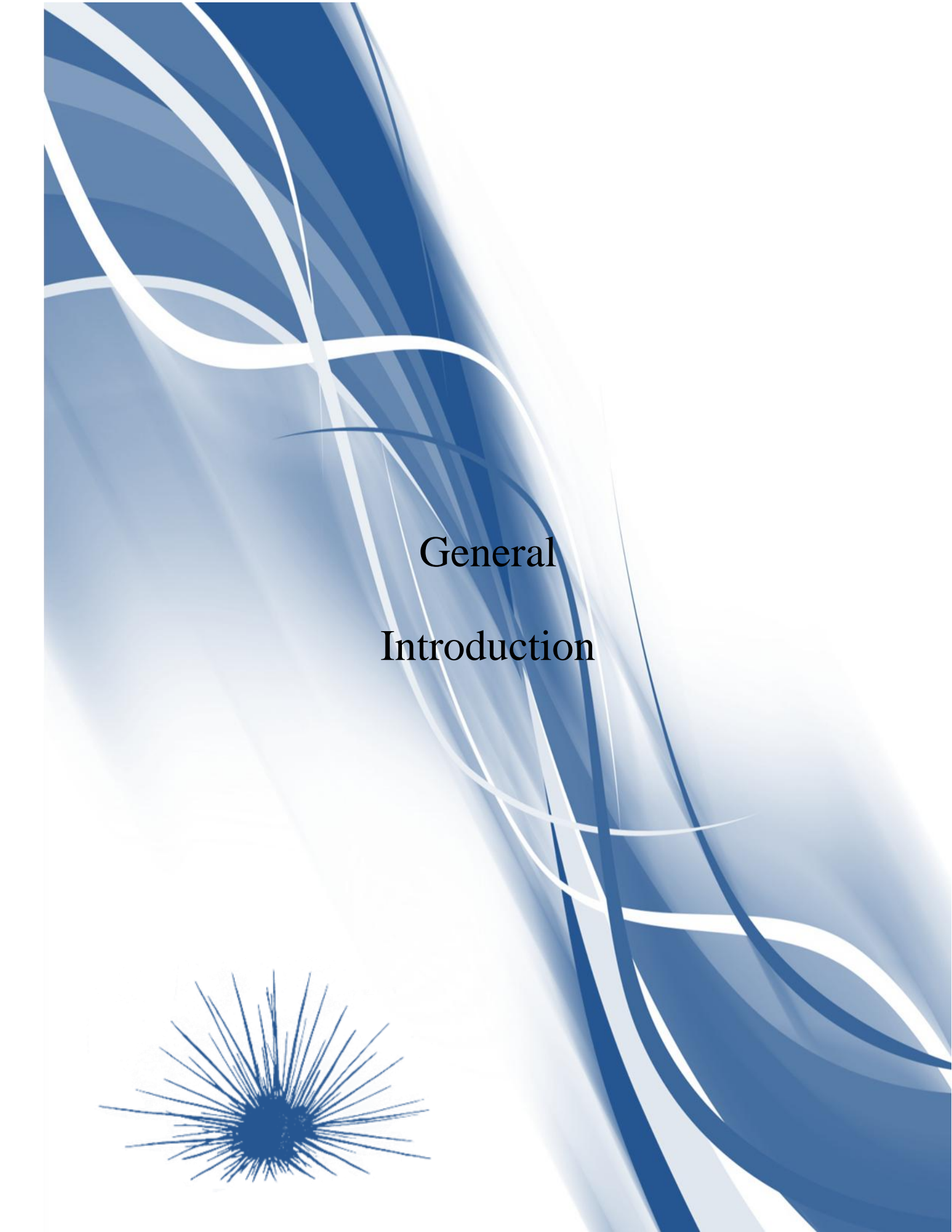
ABSTRACT

Among the key species that contribute to the stability of Caribbean coral reefs is the long spined sea urchin *Diadema antillarum* (Philippi, 1845), which together with herbivore fishes control macroalgae communities. The sea urchin *D. antillarum* suffered a mass mortality event in 1983 in the Western Atlantic, and three decades later causes of its slow recover are not evident Here, I answer for questions regarding the biology of the species: *i)* current status of this species in Puerto Rico Archipelago, *ii)* the contribution of food items in the diet of *Diadema*, *iii)* examine the apparent lack of population recovery of *D. antillarum* following the demographic modeling approach, and *iv)* the effect of two wrasses fishes in the population dynamic of this sea urchin. Changes in population structured were addressed by monitoring different populations in the northeastern coast of the Puerto Rico and Culebra. The population vital rates were evaluated using a matrix population model with size/abundance data. The contribution of algal food resources was evaluated using a stable isotope approach, whereas the impact of predators was estimated by removal of *Thalassoma bifasciatum* and *Halichoeres bivittatus* at one locality. Results indicated that *i)* the observed abundances of *D. antillarum* has not yet returned to pre-mortality levels. However, population densities of the species showed some degree of recovery when we compare with previous studies, *ii)* local recovery requires the spatial and temporal co-occurrence of high recruitment and survival rates, *iii)* results support that *D. antillarum* must be considered an omnivore in terms of assimilation. Further studies, where invertebrate samples were included, may help to clarify and quantify the real contribution of food resources in the diet of *D. antillarum*, and *iv)* the two wrasses had a strong effect in size structure and the abundance of smaller size classes.

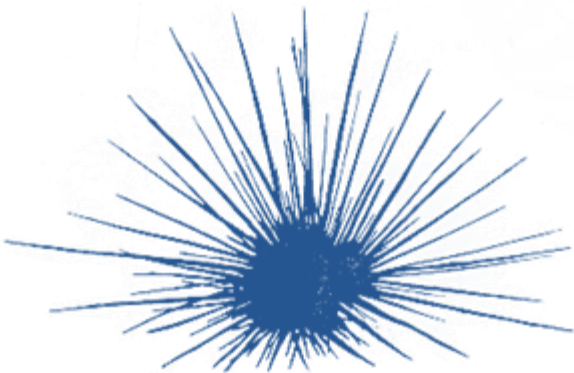
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General Introduction



The phylum Echinodermata is the largest group of extant animals that has no freshwater or terrestrial representatives. They are exclusively marine invertebrates, but some species can inhabit brackish waters. Their name derives from two ancient Greek words “*echinos*” and “*derma*”, meaning spiny skin. However, the most exclusive morphological feature of this group is the presence of a complex network of channels and superficial appendices called madreporite, and the pentameric symmetry in the adult phase, with the exception of sea cucumbers (Ruppert and Barnes 1994).

Echinoderms are represented by more than 13,000 species in the fossil record since the Precambrian, and more than 7,000 extant species (Durham *et al.* 1966, Hendler *et al.* 1995). They can be found in different biotopes from the intertidal zone to abyssal depths in all latitudes, and their presence is ecologically important in coral reef and other shallow waters ecosystems (Miller and Pawson 1989). Echinoderms are divided into five Classes: Crinoidea (sea lilies), Asteroidea (sea stars), Ophiuroidea (brittle stars), Echinoidea (sea urchins) and Holothuroidea (sea cucumbers). Six crinoids, 14 asteroids, 44 ophiuroids, 19 echinoids, and 29 holothuroids inhabit the shallow-water zone of Puerto Rico (Benavides-Serrato 2006, Rodríguez-Barreras *et al.* 2014a).

The Class Echinoidea, commonly known as sea urchins, comprises the most charismatic species among echinoderms. Sea urchins represent approximately 900 species worldwide, and more than 100 species have been reported for the Caribbean region (Hendler *et al.* 1995). They are primarily omnivorous with preference for herbivorism, and are thus important grazers in reefs ecosystems (Furman and Heck 2009, Lessios 2013). This close trophic relationship with algae and marine vascular plants makes echinoids keystone species in the maintenance and stability of shallow marine benthic ecosystems

(Sammarco 1982, Aronson and Precht 2000). The long-spined sea urchin, *Diadema antillarum* (Philippi, 1845), is one of the most charismatic and well known echinoids, due to their important role as benthic grazers in Caribbean reef ecosystems (Lessios 2013). The distribution of *D. antillarum* used to be amphiatlantic and circumtropical until 2013, when the Eastern Atlantic population was declared a new species and renamed as *Diadema africanum* (Rodríguez *et al.* 2013). In the Caribbean, *D. antillarum* populations span from Bermuda through the Gulf of Mexico, the Greater and Lesser Antilles to the North of Brazil. They can be found from the shoreline up to 40 m deep, but their common habitat is the shallow area (Hendler *et al.* 1995).

The sea urchin *D. antillarum* is classified as a generalist herbivore, feeding on algae of the genera *Padina*, *Dyctiota*, *Lobophora*, *Cystoseira*, *Halopteris*, *Galaxaura* and *Halimeda* (Solandt and Halley 2001, Tuya *et al.* 2001). Nevertheless, Rylaarsdam (1983), Karlson (1983), and more recently Rotjan and Lewis (2008) classified the species as a corallivore. This might be the result of non-selective feeding during scraping off action in the substrate according to Hendler *et al.* (1995), which may not be totally true (see Chapter II). Nevertheless, *Diadema* has other important indirect effects in the ecosystem. For instance, grazing activity favors the recruitment of scleractinian corals (Edmund and Carpenter. 2006). This sea urchin has also been proposed as a component of disease control (Nugues *et al.* 2004), whereas the spine canopy can also protect small invertebrates and juvenile fish stages against predators (Randall *et al.* 1964). Moreover, it is also considered an important source of bioerosion, contributing to particulation of rocky substrates (Hunter 1977).

Coral reefs are the most diverse and complex ecosystem on Earth, but human impacts and natural disturbances represent a serious challenge to their long-term permanence (Fabricious 2011). It is known that corals and macroalgae communities are usually substrate competitors, but macroalgae have higher growth rates (Miller *et al.* 2009). This relationship is regulated primarily by nutrient concentrations in the water column and herbivory (Hughes 1994, Carpenter and Edmund 2006). Keystone species are crucial to maintain the stability and biodiversity of ecosystems. When they fail, profound changes take place in ecosystem structure and functioning. For instance, macroalgae are opportunistic, increasing their abundances rapidly and with detrimental effects on corals when sea urchins are absent (Sammarco *et al.* 1974, Hay and Taylor 1985, Carpenter 1986). Fish of the families Scaridae and Acanthuridae, and the sea urchin *D. antillarum* are the most important herbivores in the Western Atlantic (Sammarco 1982, Tuya *et al.* 2005). In the wake of long-term overfishing, however, herbivore fishes stocks have declined, leaving *D. antillarum* as the most important algal community controller (Idjadi *et al.* 2010).

Populations of *D. antillarum* suffered a mass mortality event in 1983 in the Western Atlantic, probably caused by a waterborne pathogen that was never-isolated. The pandemic started in Panama and rapidly spread throughout the Caribbean, Gulf of Mexico, and as far as Bermuda (Lessios 1988). The die-off ended in 1984 with final mortality rates ranging from 87 to 99.9% (Hunte *et al.* 1986). This mass mortality event generated profound ecosystem changes and many reefs switched to a macroalgae-dominated state (Carpenter 1990), under the functional absence of this importance grazer (Carpenter 1981, Edmunds and Carpenter 2001). One immediate consequence of the

reduction or local extinction of *D. antillarum* was the increase of macroalgae abundances and decrease of hard corals in many Caribbean reefs (Liddell and Ohlhorst 1986). For instance, fleshy algae of the genera *Gelidium*, *Coelothrix* and *Amphiroa* sp increased their abundance weeks after the mass mortality event in Puerto Rico (Vicente and Goenaga 1984). Three decades later, *D. antillarum* is going through a slow and non-uniform recovery process where populations still exhibit low densities in many Caribbean reefs and continue to be locally extinct in others (Lessios 2005).

Several explanations have been proposed to explain this lack of recovery. Suspected factors include the “Allee” effect, larval deficit, predation, and substrate availability (Lessios 1988, Levitan 1988, Roger and Lorenzen 2008, Miller *et al.* 2009). The existence of an algae-dominated reef state affects corals and sea urchins alike. Like corals, larvae of *D. antillarum* have a similar requirement: a macroalgae free hardground. Further, secondary metabolites produced by macroalgae may reduce fertilization success, larvae survival, and settlement (Butman 1987, Hay and Fenical 1988). Adults of *D. antillarum* remove macroalgae while grazing, creating suitable condition for their own larvae and those of coral, by removing the presence of substances with negative effects on larval survival and development (Fig. 1). This emphasizes the importance of adults in creating adequate conditions for younger life stages (Roger and Lorenzen 2008).

Another potentially important factor in the observed lack of recovery is predation. Even with a well-developed defensive mechanism mediated by large thin spines, the sea urchin is still vulnerable to predation. Natural predators of *D. antillarum* include fishes from the families Balistidae, Haemulidae, Labridae and Sparidae, the spiny lobster *Panulirus*

No-take marine reserves offer one of the best “natural” experiments to study predator-prey interactions in marine ecosystems. Once predators start increasing in abundance after designation of no-fishing, sea urchin densities tend to decrease. In a study in a Bahamian marine reserve, no urchins were found inside the reserve and just a few were found around the boundaries, showing not only the effect in the delimited areas, but also the spill-over effect; suggesting that predators are leaving the reserve and impacting nearby urchin populations (Harborne *et al.* 2009). Similar results were obtained in Belize, Florida, and Cuba (Chiappone *et al.* 2002, Brown-Saracino *et al.* 2007, Martin-Blanco *et al.* 2010) On the other hand, surveys in overfished areas, such as in Jamaica, have reported the highest density values for the sea urchin (Sellers *et al.* 2009, Idjadi *et al.* 2010).

Although *D. antillarum* has been reported as feeding primarily on algae (Lewis 1964), it also ingests flowering plants and detritus (Randall *et al.* 1964). Sponges, hydroids, bryozoans, nematodes, rotifers, gastropods, bivalves, and copepods have been found in *Diadema* gut studies (Herrera-López *et al.* 2003, Hernández *et al.* 2006). Despite a number of studies related to feeding attributes, resource assimilation has been only partially explored in the genus *Diadema* (Cabanillas-Terán 2009). The idea that *D. antillarum* is not limited by food resources, because of current high abundance of macroalgae, might not always have been the case. Algal communities alone may not provide the quantity and/or quality of resources sea urchins require. Stable isotope approaches may allow us to clarify the correct trophic position of sea urchins (Wangensteen *et al.* 2011). Assimilated food items might be restricted to only few algae,

and not all the species *D. antillarum* ingest while foraging, because food resources change while in transit through the digestive system (Trenzado *et al.* 2012).

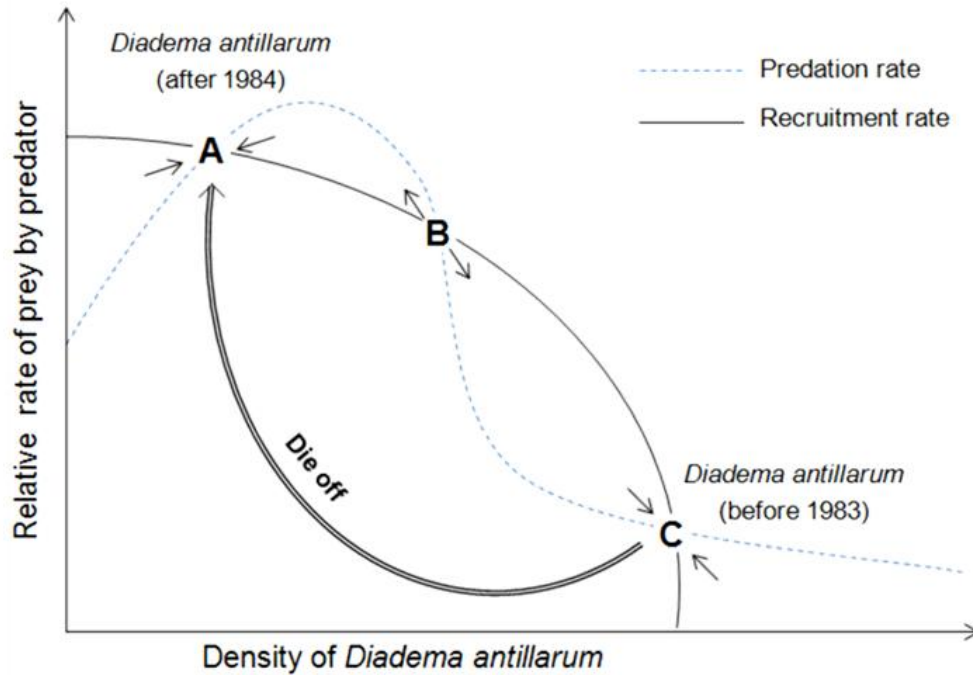


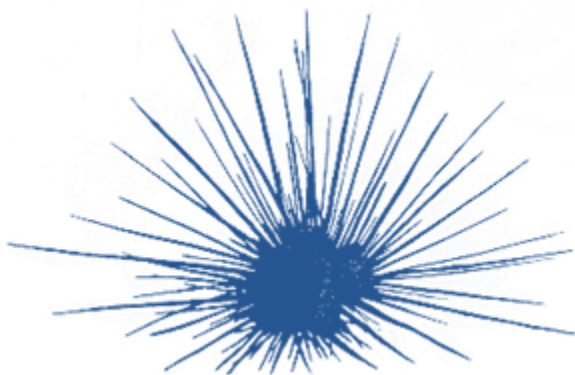
Figure 2 Model of multiple stable states for the sea urchin *Diadema antillarum* in a predator-prey system, based in curve type III of functional response. Point A represents the current population status where populations are regulated by low recruitment and high predation, whereas C represents populations after the die-off when they were controlled by food and other resources. A & C represent stable states, and B an unstable

Some chemical elements can be found as a mix of light and heavy isotopes due to differences in the number of neutrons, resulting in changes in atomic masses and not in electric or chemical properties (Cabanillas-Terán 2009). These elements are called stable because they do not radiate and maintain their unique characteristics through time. In the case of the two most commonly used isotopes, carbon and nitrogen, the information provided is different. Carbon analyses tell us what resources an organism consumes, while nitrogen indicates the trophic position of the species within the food web. Particularly for nitrogen, consumers usually incorporate preferentially ^{15}N rather than

¹⁴N. Therefore, it is possible to detect by isotopic analysis of proteins, proportional enrichment in ¹⁵N with respect to food sources (Minagawa and Wada 1984). Every organism has its own isotopic signature and there exists a relationship between the isotopic composition of the source and the receiver (De Niro and Epstein 1981). In fact, a species isotopic signature will vary according to isotopic changes in food resources (Tomas *et al.* 2006). The majority of studies in the diet of *Diadema* have focused on traditional gut content analysis (Randall *et al.* 1964, Hernández *et al.* 2006), leaving a gap of knowledge related to assimilative behavior.

The goal of this dissertation was to investigate the status of the sea urchin *D. antillarum* in the Puerto Rico Archipelago, focusing on the causes that keep its population at low densities. We set out to: i) determine the current population status of the sea urchin *D. antillarum* in Puerto Rico, ii) characterize the isotopic signatures of this sea urchin and its potential food resources, and determine its trophic position using a stable isotope approach, iii) examine the apparent lack of population recovery of *D. antillarum* following a demographic modeling approach (using capture-mark-recapture techniques), and iv) analyzed the demographic response of *D. antillarum* under low density scenarios of the fish wrasses *Thalassoma bifasciatum* and *Halichoeres bivittatus*.

Higher population densities of the sea urchin
Diadema antillarum (Philippi, 1845) linked to
wave sheltered areas in north Puerto Rico



ABSTRACT

The long-spined sea urchin *Diadema antillarum* has been the focus of multiple studies since the mass mortality event in the 1980's. The recovery of this key-herbivore in the Wider Caribbean is essential for the wellbeing of coral reefs. This study examined the population density and structure of *D. antillarum* at seven northern fringing reefs of Puerto Rico between 2011 and 2013. The total mean density of the sea urchins in northern Puerto Rico was 0.9 ± 0.3 urchin.m⁻². Densities of *D. antillarum* significantly differed among sites, but not temporally. Differences in mean sizes were significant among sites and seasons. Areas with higher densities of *D. antillarum* showed lower cover of non-calcareous algae. Wave exposure was correlated with the abundance of the sea urchin. This study indicates that the observed abundance of *D. antillarum* has not yet returned to pre-mortality levels. However, densities showed some degree of recovery when we compare with previous studies, enabling at least some degree of control on fleshy macroalgae communities. No significant changes in density occurred between 2011 and 2013, and sites with higher densities were generally located in leeward areas. The low relative abundance of small size individuals points towards recruitment limitation as an explanation for the limited recovery of *Diadema antillarum*.

INTRODUCTION

Over the past five decades coral reefs in the Caribbean have change dramatically (Gardner *et al.* 2003, Hughes *et al.* 2010, De'ath *et al.* 2012). Regional declines in coral cover have been asynchronous and due to cumulative factors such as, hurricanes, disease outbreaks, bleaching, pollution, and overfishing (Bythell *et al.* 1993, Bythell *et al.* 2000, Hughes 1994, Kramer *et al.* 2003, Hughes *et al.* 2010). One of the most dramatic shifts in community structure occurred after the massive die-off of *Diadema antillarum* (Echinodermata, Echinoidea). This species is considered a keystone herbivore in the Western Atlantic, exerting top-down control on algal abundance (Carpenter and Edmunds 2006). The long-spined sea urchin has been the focus of many studies after its epizootic event between 1983 and 1984 (Lessios *et al.* 1984), when their mortality reached up to 99.9 % at some Caribbean locations (Hughes *et al.* 1985, Hunte *et al.* 1986).

Herbivores maintain the stability and diversity of the highly-productive coral-dominated state of tropical reefs (Sammarco *et al.* 1974, Sammarco 1982, Littler *et al.* 1991, Hughes 1994, Carpenter and Edmunds 2006). *Diadema antillarum* and parrot-fishes have the greatest impact on algal abundance and the widest feeding range of algal types (Steneck 1988). Profound changes took place on reefs after the collapse of *D. antillarum* populations (Hughes *et al.* 1985), shifting the structure and function of Caribbean coral reef ecosystems (Liddell and Ohlhorst 1986). In some Caribbean reef locations, the benthic algal cover increased between 100% and 250% (Phinney *et al.* 2001). Non-calcareous algae of the genera *Gelidium*, *Coelothrix* and *Amphiroa* became more abundant in Puerto Rico (Vicente 1987). The potential role of the sea urchin as the agent facilitating the switch between the current algae-dominated state in many Caribbean reefs

to the original coral dominated one is considered a “new opportunity” for coral reefs (Knowlton 2001), given the demise of herbivorous fishes due to overfishing (Idjadi *et al.* 2010).

Pre-mortality data available for Puerto Rico is useful to understand the dynamics in the recovery process locally (Craft 1975, Rivera and Vicente 1976, Bauer 1980, Vicente and Goenaga 1984). Densities dropped from 12.7-13.8 urchin.m⁻² (Craft 1975, Bauer 1980) to less than 1.0 urchin.m⁻² (Ruiz-Ramos *et al.* 2011, Soto-Santiago and Irizarry-Soto 2013). Presently, *D. antillarum* is going through a slow and non-uniform recovery process in many Caribbean reefs where populations still exhibit low densities (Bak *et al.* 1984, Vicente and Goenaga 1984, Hughes *et al.* 1985, Hunte *et al.* 1986, Miller *et al.* 2003, Lessios 2005, Weil *et al.* 2005, Debrot and Nagelkerken 2006, Noriega *et al.* 2006, Steiner and Williams 2006, Sellers *et al.* 2009, Idjadi *et al.* 2010, Martín-Blanco *et al.* 2010 and 2011, Ruiz-Ramos *et al.* 2011, Levitan *et al.* 2014). Differences in recovery may be explained by physical factors such as wave exposure (Chollett *et al.* 2012).

Higher densities of the species have been linked to wave sheltered habitats (Clemente and Hernández 2008, Hernández *et al.* 2008). Leeward areas seem to favor higher densities of *Diadema* and seems to be an important oceanographic factor modeling population dynamics (Debrot and Nagelkerken 2006). The effect of unidirectional hydrodynamic forces on *D. antillarum* could be explained because its morphological features do not allow a large adhesive area to attach, being considered weakly resistant to wave exposure (Tuya *et al.* 2007).

On the other hand, the recovery of *D. antillarum* populations at some Caribbean sites has been related to reductions in macroalgae cover and increase of the recruitment for reef-

building corals (Bellwood *et al.* 2004, Carpenter and Edmunds 2006, Idjadi *et al.* 2010, Soto-Santiago and Irizarry-Soto 2013). It is crucial to assess the current status of *D. antillarum* populations a decade after the last available data in order to monitor the population dynamics of this important herbivore. The main objectives of this study were to: (i) determine the current density of *D. antillarum* and compare our results with previous studies to determine whether *D. antillarum* populations have recovered, (ii) analyze spatial and temporal changes in density and size structure, and (iii) explore the relationship among the abundance of *D. antillarum*, and wave exposure, and some of main reef functional groups (calcareous algae, fleshy macroalgae, turf, and corals).

METHODS

Study area- surveys were conducted between August of 2011 and August of 2013 at seven shallow-water fringing reefs (1- 3 m deep). The sites were Cerro Gordo-A (CGA- 18°16'51.40"N, 65°17'12.21"W), Cerro Gordo-B (CGB- 18°28'53.78"N, 66°20'25.12"W), Isla Verde (IVD- 18°26'49.79"N, 66°0'49.88"W), Luquillo (LQY- 18°23'18.46"N, 65°43'5.52"W), Tamarindo-1 (TM1- 18°18'55.29"N, 65°19'5.82"W), Tamarindo-2 (TM2- 18°19'0.78"N, 65°19'2.60"W), and Melones (MLN- 18°18'15.70"N, 65°18'42.27"W) (Fig. 1). The first four sites are characterized by a very narrow shelf and high energy sandy beaches, due to the effect of northeast trade winds and North Atlantic winter storms. Due to the high annual precipitation levels and the discharge of rivers (Williams *et al.* 2013), high sediment loads are common in CGA, CGB, IVD and LQY. On the other hand, TM1, TM2, and MLN are located in Culebra Island. This island has no rivers, precipitation is low, and it is characterized by volcanic and intrusive rocks,

with some limestone deposits, whereas sites along the north coast (CGA, CGB, IVD and LQY) are made up by carbonate rocks (U.S.G.S. 1996).

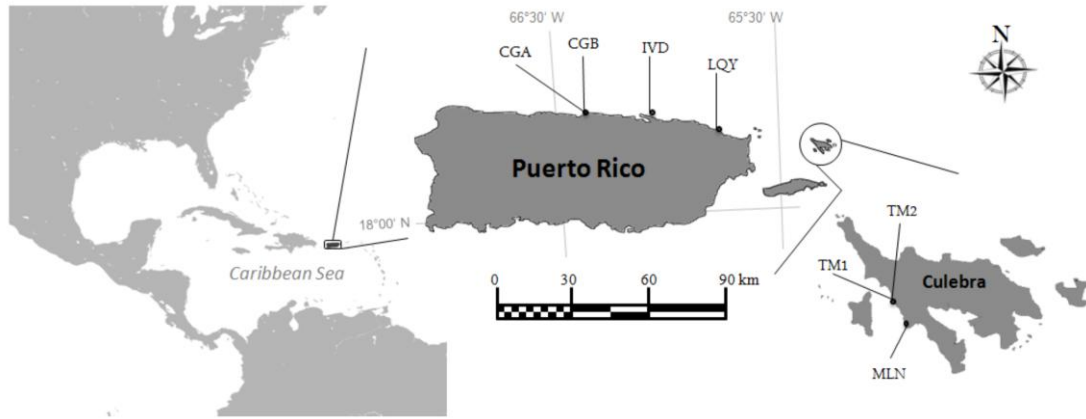


Figure 1. Study area and sampling sites in Puerto Rico. Cerro Gordo-A (CGA), Cerro Gordo-B (CGB), Isla Verde (IVD), Luquillo (LQY), Tamarindo-1 (TM1), Tamarindo-2 (TM2), and Melones (MLN).

Survey methodology- Mean densities were estimated using a belt-transect methodology (Sellers *et al.* 2009). At each site, eight fixed transects of 10 m² (5 m x 2 m) were placed parallel to the coast and separated 10 m from each other. All individuals within transects were counted. All crevices and small holes were carefully inspected to avoid missing any individuals. Field sampling was carried out every six months. We selected August and February based on differences in local precipitation and temperature (NOAA Coral Reef Watch 2000), because both abiotic factors have some influence on echinoid population dynamics (Lugo-Ascorbe 2004, Williams *et al.* 2009). With the use of a caliper, we measured the test diameter of fifty sea urchins, which were collected randomly every season by site (caliper error \pm 0.05 mm). We divided populations in three size categories: small: < 40.0 mm; medium: 41.0- 60.0 mm; and large: > 60.0 mm following Miller *et al.*, (2003).

Substrate complexity, cover and wind analysis- The rope-and-chain method was used to measure the substrate complexity at all sites. It was expressed as the ratio between the total length of a chain and the length of the same chain when molded to the reef surface [Complexity Index = total length of the chain / total distance when mold the bottom]. The range varied from 1 (flat surface) to higher values indicating more complex topography (Alvarez-Filip *et al.* 2009). Five PVC tube quadrats of 25 cm² per transect were photographed and analyzed using CPCe-4.1 (Kohler and Gill 2006). We selected 25 points randomly per picture and estimated percent cover of the following categories: fleshy macroalgae (NCAL), calcareous algae (CAL), turf, and live coral (LCOR), using only data from August 2012. Furthermore, we analyzed the relationship between the abundance of *D. antillarum* and the wave exposure based on the chronic stress map given by Chollet *et al.* (2012). Wave exposure values varied in a scale from 1 (low) to 9 (high). Sites in Culebra (TM1, TM2, MLN) were under moderate wave action (5.5), LQY occupied an intermediate position with 7.0, whereas IVD, CGA and CGB were the most affected sites with 7.9. We re-evaluated CGB because Chollett *et al.* (2012) did not reflect the existence of a natural protection against wave exposure. Culebra sites have a similar protection against high wave action due to the presence of Luis Peña Key in front of these sites. Because of this, we decreased the category of this site from 7.9 to 5.5.

Statistical analysis- We fitted a two-way repeated measures ANOVA model to detect differences in mean density among sites. The model incorporates time as fixed factor, and transect (fixed factor) nested inside site (random factor). Mean size was analyzed using a two-way ANOVA model with time as fixed factor, and site as random one. Fixed effects were tested using F tests; models involving different random effects were compared using

likelihood ratio tests. Mean density data followed a normal distribution. A power transformation was applied for reaching normality and homogeneity of variance (Box and Cox 1964). Result suggested the square (not the square root) for mean size (suggesting a normal distribution for the area of the transversal section). We ran a *post-hoc* Bonferroni test for multiple comparisons of means for density and size (Zar 2010). Pearson's correlation test was used to determine the relationship between the mean density of *D. antillarum* and non-calcareous algae (fleshy macroalgae), coral, and substrate complexity, but not turf. Potential differences among small, medium and large categories for each site and season were analyzed by fitting log-linear models. Furthermore, we run a non-parametric Spearman correlation test between wave exposure and the mean density of *D. antillarum*. Statistical analyses were run in the free-license statistical program R-3.0.1 with a *pval*: 0.05 and 0.01, <http://www.r-project.org/> (R Core Team 2013). Packages MASS and nlme were used for estimation of power transformations, and for complex repeated measured analysis of variance respectively (Venables and Ripley 2002, Pinheiro and Bates 2013).

RESULTS

Spatial and temporal abundance- Overall mean density (\pm 95% confidence interval) of *D. antillarum* in Puerto Rico was 1.148 ± 0.345 ind m^{-2} at the beginning of the study, and 0.946 ± 0.295 ind m^{-2} at the end of the study. The highest density recorded was 1.398 ± 0.342 urchin. m^{-2} in February 2012, whereas a minimum of 0.946 ± 0.295 urchin. m^{-2} was in August 2013. Sites were generally stable temporally in mean density, except in CGA and LQY, where densities increased 0.85 and 0.90 urchin. m^{-2} from August of 2011 to

February of 2012 respectively. However, temporal differences were not detected among sites (Tab. 1).

Table 1. Two-way repeated measure analysis of variance on the abundance (a), and the two-way anova test on the size of the sea urchin *Diadema antillarum* (b). * this value comes from a Chi² test that compares models with and without site as a random effect.

	a) Mean density			b) Mean size		
	df	F	p-value	df	F	p-value
Site (s)	-	-	0.001*	6	263.06	<0.001
Time (t)	4	1.261	0.288	4	1.593	0.209
Site x time	30	1.218	0.223	24	3.192	<0.001

Spatial differences were found (Tab. 1). These differences among sites were related with the low densities reported in IVD and CGB (Fig. 2). Differences were found between CGB and CGA (Bonferroni, $p=0.025$), and TM2 (Bonferroni, $p=0.004$); and also between IVD and CGB (Bonferroni, $p=0.033$) and TM2 (Bonferroni, $p=0.006$). During this study mean densities at CGB, TM2, and MLN were above $1.0 \text{ urchin.m}^{-2}$ (Fig. 2). At TM2 the maximum peak of abundance was $2.402 \pm 0.624 \text{ urchin.m}^{-2}$ during February of 2012, whereas at IVD and CGA reported densities were below $0.50 \text{ urchin.m}^{-2}$.

Size structure- mean size of *D. antillarum* fluctuated throughout the study with maximum values during summers ($6.51 \pm 0.14 \text{ cm}$) and minimums ones during winter ($6.23 \pm 0.15 \text{ cm}$; Fig. 3). However, seasonal differences were not significant (Table 1). The mean size peaked at IVD during February of 2013 with $7.91 \pm 0.77 \text{ cm}$; while TM2 reached the lowest mean size of $4.04 \pm 1.08 \text{ cm}$ during February of 2012. We found spatial and site-time interaction differences on size (Tab. 1). Spatial differences were related mainly with TM2, the site with the lowest mean size, due to the high abundance of medium and small

sizes found in this site (Bonferroni, $p < 0.00001$), and also among IVD and the other stations (Bonferroni, $p < 0.0001$; Fig. 3).

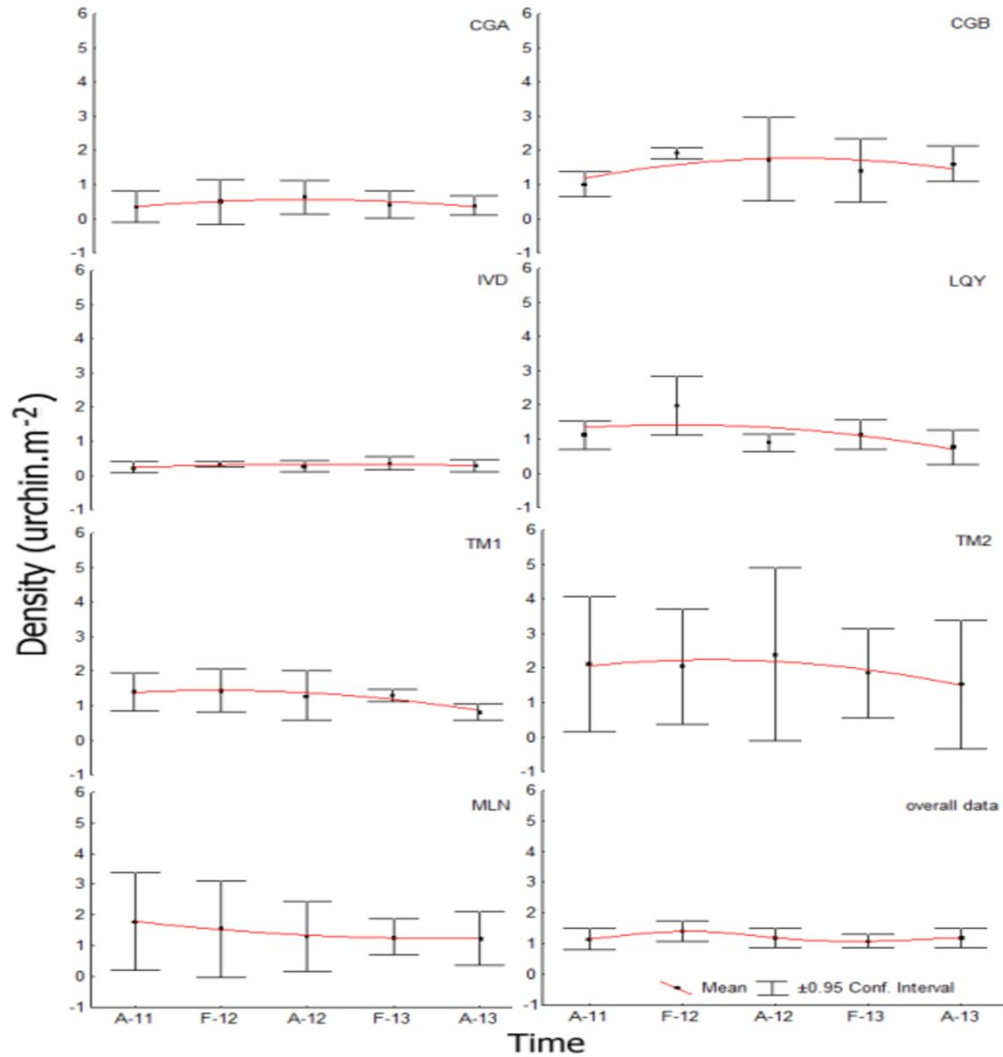


Figure 2. Spatial and temporal density of *Diadema antillarum* in Puerto Rico from August 2011 to August 2013 at CGA, CGB, IVD, LQY, TM1, TM2 and MLN (see methods for acronyms). The X axe represents time with A-11 (August-2011), F-12 (February-2012), etc. The Y axe is the mean density of *D. antillarum* (urchins.m⁻²). Bars represent 95% of confidence interval of the mean and red line represents a polynomial fit. Last graph shows the overall temporal change of mean density using pooled data.

Small individuals (< 40 mm) were the less common size class, contributing with 14 % of 1,534 echinoids measured during two years at all sites. The exception was TM2, where we found an unusual population size structure, dominated by medium and small size classes (Fig. 3). Small sea urchins represented up to 44% during August 2011, whereas large sea urchins did not reached 10 % of abundance at any time in TM2. When we considered new recruits (< 25 mm) as a separate size class, they were only 1.10 % of the overall individuals measured during the whole study at all sites; and the majority of them were reported during August 2011. No juveniles were detected at IVD, CGB and TM1, whereas the two sites with the greatest number of juveniles were TM2 (8%) and CGB (4%) during August of 2012.

The large size class (> 60 mm) was the most abundant in space and time, followed by the medium size (41 - 60 mm); while the small category (< 40 mm) was the scarcest one. The cohort distribution analysis by fitting log-linear models showed that population structures changed according to site, and these changes were affected by the season ($p = 2.59 \times 10^{-8}$). Sites located near each other such as CGA/CGB and TM1/TM2 showed different size structure. Medium and small cohorts were more abundant at CGB and TM2 while large cohort was dominant at CGA and TM1.

Substrate complexity, cover, and wave exposure- Substrate complexity varied from 1.06 ± 0.02 units at LQY to 1.37 ± 0.16 units at MLN. Fleshy macroalgae (NCAL) varied from a minimum of 1.8 ± 2.75 % in TM2, to a maximum of 43.6 ± 13.21 % in CGA (Fig. 4). Turf showed a similar pattern, with a minimum of 1.45 ± 3.69 % at TM2 and a maximum of 18.18 ± 7.01 % at CGA. Calcareous algae were more abundant at sites with both less fleshy macroalgae and turf (Fig. 4). The lowest percent of calcareous algae was

found at CGA with 1.09 ± 1.87 %, while TM1 displayed the highest percent with 10.18 ± 10.93 %. Coral cover was low at CGA and IVD (< 10 %), but greater than 15% at the other sites, peaking at TM2 (29.09 ± 12.15 %, Fig. 4). We found a significant inverse relationship between the abundance of *D. antillarum* and fleshy macroalgae (Pearson, $r^2 = 0.909$, $r = -0.954$, $p = 0.0009$). We found low correlation between the abundance of *D. antillarum* and coral cover (Pearson, $r^2 = 0.305$, $r = 0.416$, $p = 0.0093$), and no significant relationship between sea urchin abundance and substrate complexity (Pearson, $r^2 = 0.541$, $r = 0.295$, $p = 0.179$). In addition, mean density of *D. antillarum* was correlated with wave exposure ($r^2 = 0.519$, $r = -0.580$, $p = 0.0003$).

Mean density of *D. antillarum* was correlated with wave exposure followed the wave action map on Chollett *et al.* (2012) ($r = -0.58$, $p = 0.0003$). However, the wave exposure map did not take into account the existence of small key close to CGB that protect this site from extreme wave action. We adjusted the value of CGB (from 7.9 to 5.5) and obtained a higher correlation ($r = -0.80$, $p = 0.00001$).

DISCUSSION

There has been a slow and patchy recovery of *D. antillarum* populations throughout the Caribbean region (Miller *et al.* 2003, Weil *et al.* 2005, Steiner and Williams 2006, Debrot and Nagelkerken 2006, Brown-Saracino *et al.* 2007, Miller *et al.* 2007, Harborne *et al.* 2009, Seller *et al.* 2009, Idjadi *et al.* 2010, Martín-Blanco *et al.* 2010 and 2011, Ruiz-Ramos *et al.* 2011, Soto-Santiago and Irizarry-Soto 2013, Levitan *et al.* 2014). Recently, relative high densities have been reported in Jamaica, with up to 4.77 urchin.m⁻² (Idjadi *et*

al. 2010), whereas Florida reported a maximum of 0.33 urchin.m⁻² after more than two decades of the die-off (Chiappone *et al.* 2008).

The available historical data related to *D. antillarum* populations is fragmented and scarce in Puerto Rico, limiting our understanding of the recovery dynamics of this important herbivore. Populations of the sea urchin dramatically collapsed from 10 urchin.m⁻² to close to zero after the die-off occurred in 1984 in Puerto Rico (Back *et al.*

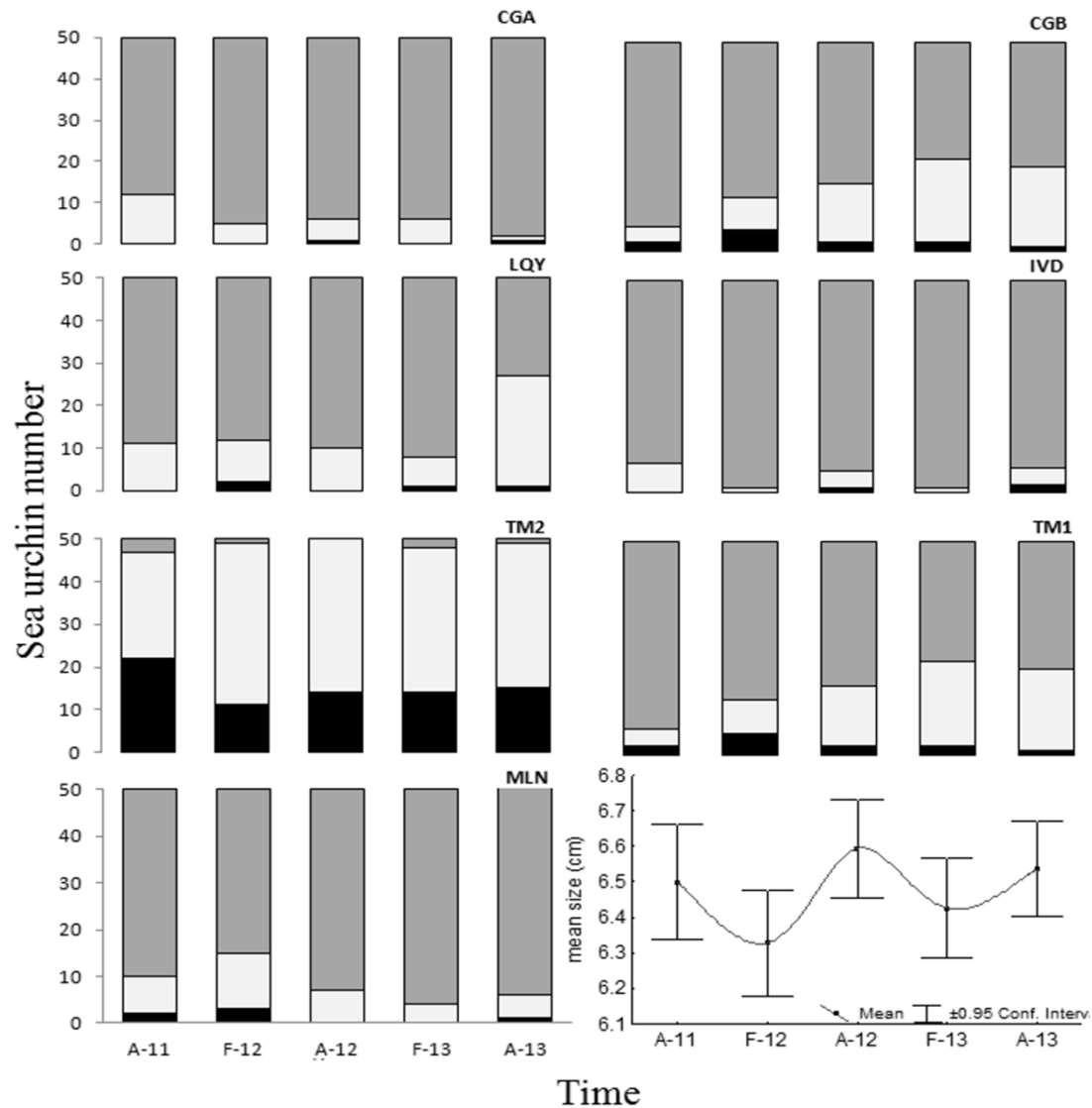


Figure 3. Seasonal size class distribution of *Diadema antillarum* at CGA, CGB, IVD, LQY, TM1, TM2 and MLN in Puerto Rico (see methods for acronyms). Black bar: small size (< 40 mm), white: medium size (41-60 mm), and dark gray: large size (> 60 mm). Last graph shows the temporal variation of mean size of *Diadema antillarum* of all sites pooled.

1984). Bauer (1980) reported densities of $13.8 \text{ urchin.m}^{-2}$ in San Juan few years before the mass-mortality. Current densities remain far from pre-mortality levels (Weil *et al.* 2005). Twenty years after the mass mortality, Ruiz-Ramos *et al.* (2011) reported *D. antillarum* densities of less than 1.0 ind m^{-2} at 26 localities around Puerto Rico. We surveyed one of their sites and found a small increase in *D. antillarum* density, from $0.04 \text{ urchin.m}^{-2}$ (Ruiz-Ramos *et al.* 2011) to $0.36 \pm 0.213 \text{ urchin.m}^{-2}$. A similar pattern was seen at TM1 where density increased from $0.67 \text{ urchin.m}^{-2}$ reported in 2004 (Ruiz-Ramos *et al.* 2011) to $1.09 \pm 0.411 \text{ urchin.m}^{-2}$ nine years later. Average densities close to 1 urchin.m^{-2} are not far from desired densities between $2\text{-}3 \text{ urchin.m}^{-2}$ to control fleshy macroalgae (Steiner and Williams 2006). However, the pre-mortality densities for *D. antillarum* in the north coast of Puerto Rico about $10\text{-}14 \text{ urchin.m}^{-2}$ (Craft 1975, Back *et al.* 1984) was excessive and could have been causing significant erosion of reef hardground (Hunter 1977).

Differences in recovery may be explained by local factors such as predation, overfishing, sedimentation, and wave exposure (Harborne *et al.* 2009, Seller *et al.* 2009, Ruiz-Ramos *et al.* 2011, Chollett *et al.* 2012). Wave exposure may model population dynamic at local scale (Chollett *et al.* 2012), and it seems to be controlling population abundances of *D. antillarum* in northeastern Puerto Rico Archipelago. *Diadema* sp. is considered weakly resistant to unidirectional hydrodynamic forces, because their morphological feature does not allow a large adhesive area to attach to the substrate (Tuya *et al.* 2007). Our analysis based on the wave exposure map of Chollet *et al.* (2012) supports that leeward sites (TM1, TM2, CGB, and MLN) tend to have greater densities of *D. antillarum* ($r = -0.80$, $p = 0.00001$). These four sites are protected from high wave action because of the

existence of small keys in front of them. On the contrary, IVD and CGA do not have any physical protection against wind/wave action, and are exposed to more wave action, especially during winter season. In consonance, lower densities of *D. antillarum* were reported in these two sites. This result agrees with previous studies where higher densities of *Diadema* sp. have been linked with wave sheltered areas (Debrot and Nagelkerken 2006, Clemente and Hernández 2008, Hernández *et al.* 2008).

Size structure of *D. antillarum* changed seasonally with a reduction of mean size observed during the winter season (Fig 3). Previous studies in Puerto Rico have found temporal variability in settlement and recruitment that have an influence in mean sizes (Lugo 2004; Williams *et al.* 2009). The species usually has a maximum spawning peak between October and November (Lessios 1981), with permanence in the water column between 35 to 75 days (Leber *et al.* 2008). A reduction of mean size on February could be consequence of recruitment. Small individuals are usually more abundant during winter in the Caribbean according to Hunte and Younglao (1988); however, we did not observe a clear seasonal pattern related with the abundance of small individuals among sites (Fig. 3). Furthermore, the presence of new recruits (< 25 mm) was limited to 1.7% of the 1,534 sea urchins measured during the whole study. This suggests a serious deficiency of recruits on Puerto Rico, and points towards recruitment limitation as a possible explanation for the lack of recovery of the species in Puerto Rico. Current low densities of *D. antillarum* may be having a negative indirect effect on their recovery. A study conducted on the sister species *Diadema africanum*, former *Diadema* aff. *antillarum* (Rodríguez *et al.* 2013), showed a positive relationship between the abundance of adults and the abundance of juveniles (Tuya *et al.* 2006).

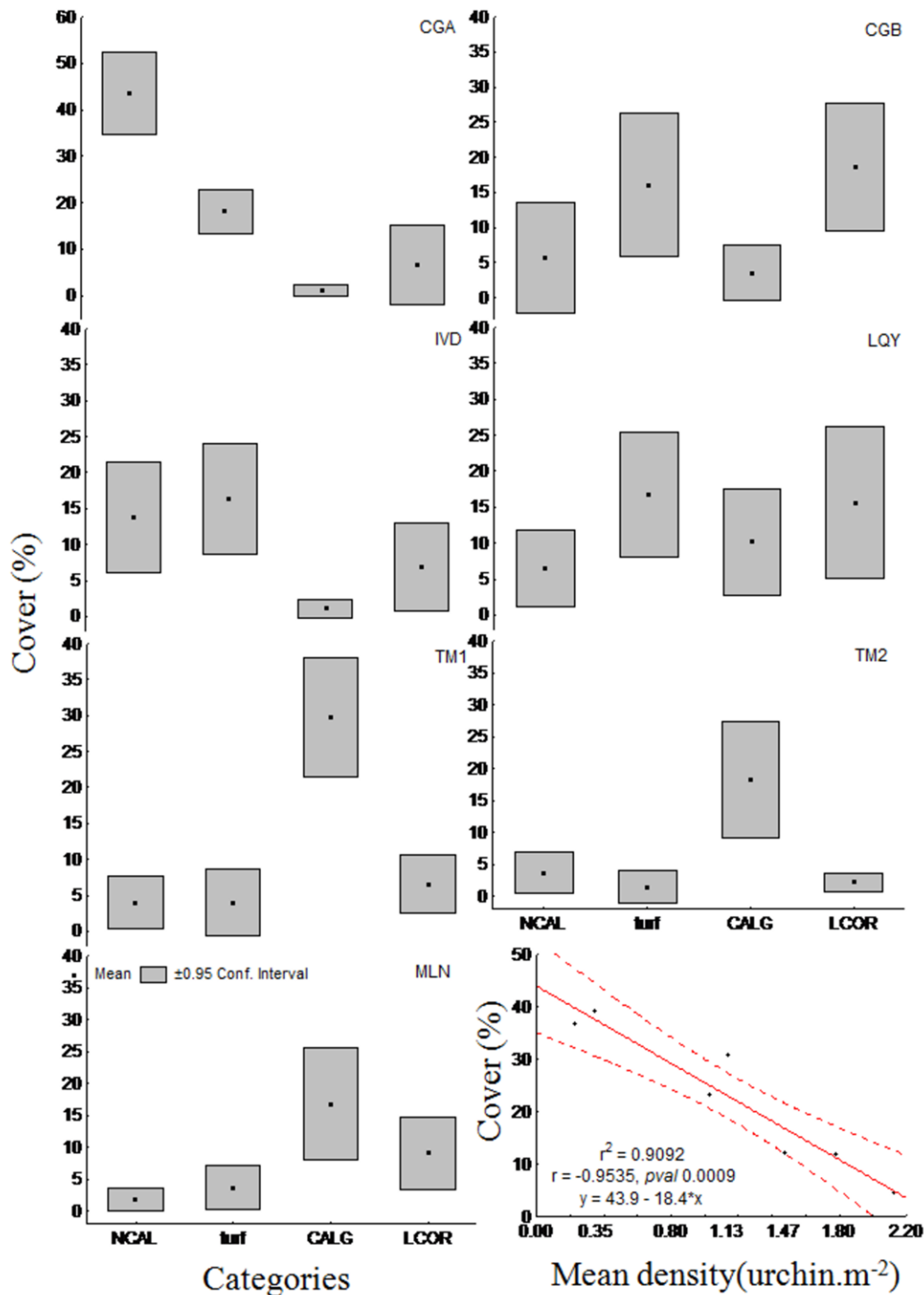


Figure 4. Spatial variation of the percent cover of fleshy macroalgae (NCAL), turf, calcareous algae (CALG), and live coral (LCOR) at CGA, CGB, IVD, LQY, TM1, TM2 and MLN in Puerto Rico (see methods for acronyms). Last graph shows the relationship between density of *Diadema antillarum* and percent cover of fleshy macroalgae (NCAL). Box represents 95% of confidence interval of the mean.

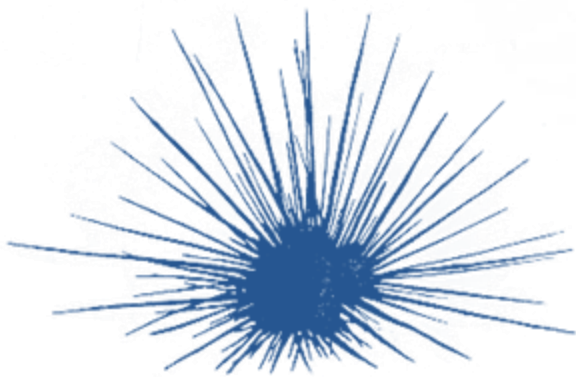
Coral cover and calcareous algae tend to increase, whereas non-calcareous become less abundant when the echinoid is present (Edmunds and Carpenter 2001, Carpenter and Edmunds 2006). Coral coverage has declined worldwide while non-calcareous seaweeds have increased (Fabricius 2011). Mean live coral cover reported in this study was less than 25%, but it was even lower at sites with low densities of *D. antillarum* (Fig. 2 and 4). Our results are consistent with others studies conducted in the Caribbean (Williams and Polunin 2001, Myhre and Acevedo-Gutiérrez 2007, Martín-Blanco *et al.* 2011) where the abundance of the black sea urchin correlates negatively with cover of fleshy macroalgae. However, a recent study found an increment of algal dominance in coral reef systems despite of the presence of *D. antillarum* (Lacey *et al.* 2013). This apparent lack of relationship could be a consequence of the current low densities, insufficient to maintain a successful control of seaweeds.

Sea urchin adults not only remove macroalgae while grazing; they create adequate conditions for larval settlement (Butman 1987, Karlson and Levitan 1990, Miller *et al.* 2007, Rogers and Lorenzen 2008). *Diadema* larvae require a hardground free of non-calcareous algae. The presence of secondary metabolites produced by algae, such as tannins and phenols, can affect the fertilization success, larvae survival, and the detection of the sea urchin adult chemical signals (Pennington 1985, Butman 1987, Hay and Fenical 1988). The observed negative correlation between sea urchin density and fleshy macroalgae (Fig. 4), which supports the importance of the species as seaweed controllers (Edmunds and Carpenter 2001). The maintenance of relative high abundance of fleshy macroalgae in CGA and IVD (Fig. 4) could preclude *D. antillarum* settlement, and consequently, the recovery of this important herbivore.

The necessity of refuge against predators offers a congruent explanation for the existence of a positive relationship between the abundance of sea urchins and substrate complexity (Tuya *et al.* 2004). It is known that higher environmental complexity provides better refuge for *D. antillarum* (Valdez and Villalobos 1978, Weil *et al.* 2005). However, the lack of correlation between mean density and substrate complexity found here, and in other recent study (Martín-Blanco *et al.* 2010) suggests a nonlinear response between rugosity and urchin abundance. At a certain threshold of abundance, habitat features should determine site-to-site variability of population densities, but at low densities there would be available living space for all individual even on less complex hardgrounds.

To conclude, the observed abundance of *D. antillarum* has not yet returned to pre-mortality levels. However, populations densities of the species showed some degree of recovery when we compare with previous studies, enabling at least some degree of control on fleshy macroalgae communities (Fig. 4). No significant changes in density occurred between 2011 and 2013, and sites with higher densities were generally located in leeward areas. Further studies are required to understand how this factor may affect the population dynamics of this important herbivore across a wave gradient. Southern coasts of the Island should be considered given it is sheltered against winter storms. The low relative abundance of small size individuals is worrisome and points towards recruitment limitation as an explanation for the limited recovery of *D. antillarum* in north Puerto Rico Archipelago after three decades of the die-off event.

Assimilative omnivory displayed by the
sea urchin *Diadema antillarum*



ABSTRACT

The sea urchin *Diadema antillarum* has been characterized as a generalist grazer; however, little is known about the species isotopic characterization, and its assimilation process in the Western Atlantic. The main goals of this study were to characterize the isotopic signatures of the sea urchin *D. antillarum*, its potential food resources, and determine the trophic position of *Diadema* using a stable isotopes approach. The study was conducted during February and October of 2013 at four shallow reefs of Puerto Rico Archipelago, where we collected samples of the sea urchins and its potential algal food resources. Temporal and spatial differences were not found on *D. antillarum* isotopic signatures of $\delta^{13}\text{C}$, but we found differences in $\delta^{15}\text{N}$ among periods and collection sites. The Trophic enrichment factors (TEFs) ranged from 2.35 ± 0.11 to 3.24 ± 0.17 , and exhibited spatial and temporal significant differences. The lack of congruent polygons found in this study, and high TEFs reinforce the importance of invertebrates as potential resources of nitrogen. We conclude that our results support that *D. antillarum* must be considered an omnivore in terms of assimilation. Further studies, where invertebrates were included, will help to quantify the contribution of the whole food sources of *D. antillarum*.

INTRODUCTION

Herbivory and nutrient availability have a direct effect in the composition and abundance of seaweed communities (Lapointe *et al.* 2004). The overabundance of macroalgae has been related to eutrophic conditions, and to the lack of herbivores (Sterner 1986, Sterner and Hessen 1994). Nevertheless, their excess should not be interpreted as an unlimited resource for herbivores. Several species of seaweeds contain high levels of undigestible and chemical deterrents to reduce grazing activity (Hay and Fenical 1988, Duarte 1990, Carpenter 1990). These secondary metabolites such as phenols and tannins may affect the consumer digestive processes by hindering assimilation (Paul and Hay 1986). On the other hand, marine trophic relationships are more complex than usually thought. A simple list of food resources based on stomach content does not identify which items are being assimilated by a consumer (Phillips 2014).

Primary producers and consumers are part of a complex trophic system where changes in the sources isotopic signatures have an effect in the signal of the consumers (Fry and Sherr 1984). There are many studies on feeding behavior and gut content but the assimilation process has been little explored in echinoids (Rodríguez 2003). The stable isotope approach has become in an important tool in ecological analysis of diets (Parnell *et al.* 2010, Boecklen *et al.* 2011). The characterization and later analysis of the isotopic signals of carbon, nitrogen, sulfur, oxygen, and hydrogen, has allowed a better comprehension of how nutrients migrate through food-webs, the assimilation efficiency per level and the trophic position of the organisms (Fry and Sherr 1984, Peterson and Fry 1987, Vander-Zanden and Rasmussen 2001, Phillips and Gregg 2003), the origin and

transformation of the ingested organic matter (Peterson *et al.* 1985), and the coexistence of organisms with similar trophic behavior (Vanderklift and Kendrick 2004, Vanderklift *et al.* 2006).

Stables isotopes analyses have allowed the development of new tools for a better understanding of the assimilative behavior. For instance, mixing models are based in the premise “you are what you eat” (De Niro and Epstein 1976), and employ Bayesian algorithms to assess the proportions of food resources assimilated by the consumer (Phillips *et al.* 2014); whereas trophic level allow to determine the exact position of a given species in the ecosystems (Hobson and Welch 1992). This powerful tools has been used to elucidate diet plasticity, differential assimilation of resources, association with biosynthetic pathways, etc. (Villinski *et al.* 2004, Tomas *et al.* 2006, Wing *et al.* 2008). Likewise, as nutrients migrate through the food web, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values of the consumers (mixture) are related with the signal of their food resources (Phillips 2014). However, isotopic signals may fluctuate due to changes in metabolic activity, seasonal changes, or environmental differences caused by human disturbances (Cole *et al.* 2004, Pruett *et al.* 2006, Baeta *et al.* 2009).

The assimilation process and the digestive enzymes of echinoids have been indeed explored in several works (Lewis 1964, Klinger 1984, de Oliveira 1991, Trenzado *et al.* 2012). Nevertheless, little is known about the isotopic characterization of sea urchins in the Western Atlantic. Stable isotopes have also allowed clarifying the trophic position of sea urchins, sometimes going against pre-established concepts (Wangenstein *et al.* 2011). Echinoids are one of the most important herbivores of shallow-water reefs in the Western Atlantic, capable of controlling seaweeds communities (Carpenter 1990). One of them,

the once abundant *Diadema antillarum* has been target of several studies because its capacity to control algal communities (Phinney *et al.* 2001), and classified as a generalist grazer (Solandt and Halley 2001, Tuya *et al.* 2001). Nevertheless, the finding of small invertebrates during gut content analysis induces doubts about the real assimilative behavior of *D. antillarum* (see Herrera-López *et al.* 2003, Hernández *et al.* 2006). Therefore, the main goals of this study were to characterize the isotopic signatures of the sea urchin *D. antillarum*, its potential food resources, and determine its trophic position using a stable isotopes approach.

METHODS

Study site- Surveys were conducted during February and October of 2013 at four shallow-water reef crests (1-2 m depth) of Puerto Rico and Culebra Island: Cerro Gordo (CGD, 18°16'51.40"N, 65°17'12.21"W), Luquillo (LQY, 18°23'18.46" N, 65°43'5.52" W), Tamarindo (TMD, 18°18'55.29"N, 65°19'5.82"W), and Melones (MLN, 18°18'15.70"N, 65°18'42.27"W) (Fig. 1). The northern coastline of Puerto Rico is characterized by a very narrow shelf and high energy sandy beaches, due to the action of northeast trade winds and North Atlantic winter storms. Due to the high annual precipitation levels and the discharge of rivers (Williams *et al.* 2013), high sediment discharges are common in the north. On the other hand, Culebra has no rivers and precipitation is low. TMD and MLN are characterized by volcanic and intrusive rocks with some limestone deposits, whereas CGD and LQY are made up by carbonate rocks (U.S.G.S. 1996). See Rodríguez-Barreras *et al.* (2014a) for more details on site description.

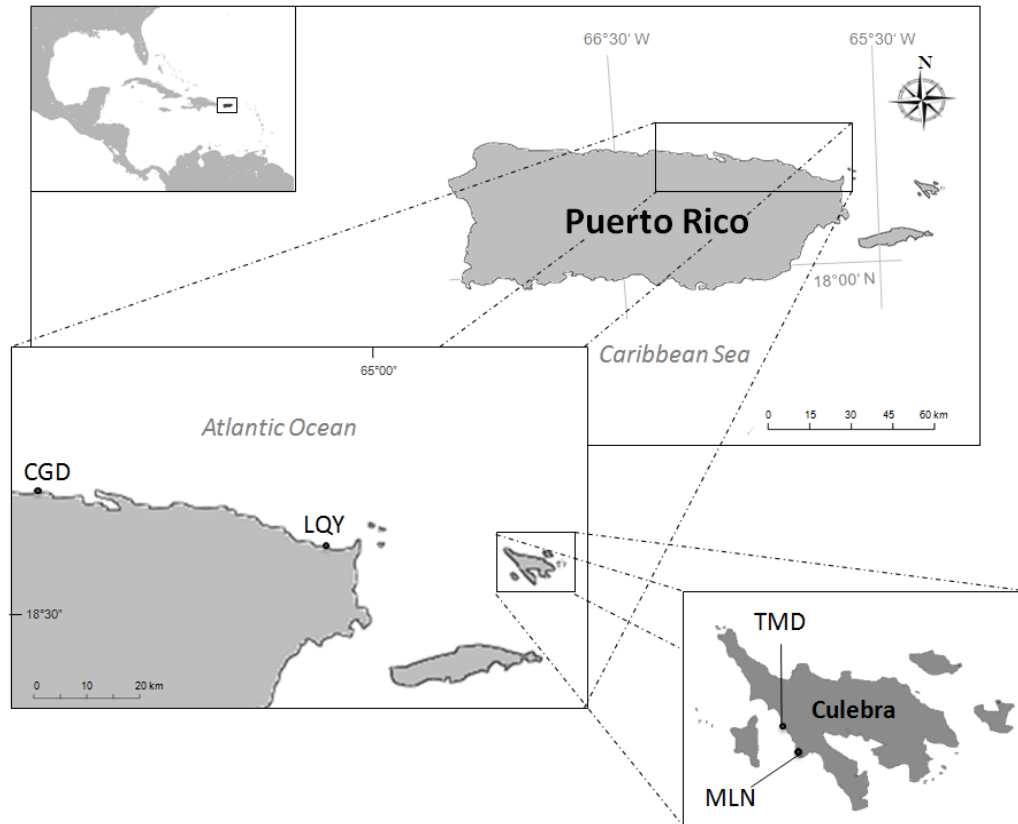


Figure 1 Study area and sampling sites in Puerto Rico: Cerro Gordo (CGD), Luquillo (LQY), Tamarindo (TMD), and Melones (MLN).

Collection and processing- Algal biomass and relative abundance were measured using ten quadrats of 25 x 25 cm per site and season. Substrate inside each quadrat was scrapped carefully and all seaweeds removed, put in separated bags, and frozen for later analysis. Macroalgae were identified to the lowest possible taxonomic level using Littler and Littler (2000), and divided in two categories: fleshy and coralline algae. Seaweeds were dried in an oven for 48 hours at 60 °C, and weighed in a digital balance (error= 0.0001 g). For isotopic analysis we collected five adult sea urchins of *Diadema antillarum* per site and season. Only adults (> 40.0 mm mean test diameter) were selected to avoid a potential life cycle stage effect. We also collected samples of all macroalgae species within a radius of 10 m from collected sea urchins. All samples (sea urchin and

macroalgae) were frozen and transported to the laboratory for later analysis. Once defrosted, algae with high carbonate content such as *Padina* spp., and *Jania* spp, were washed previously in a solution of formic acid-sodium formate buffer to remove the excess of carbonates before drying (Cabanillas-Terán 2009). For sea urchins, the Aristotle's lantern muscles were removed and no lipid extraction was necessary because $C/N < 3.5$ (Post *et al.* 2007). All samples were then oven-dried for 48 hours at 60 °C. Once dried, they were grounded to a fine powder using Retsch M-200 frequency grinder and weighed. A sample of 0.9 - 1.5 mg for muscle and 4.0 - 4.5 mg for macroalgae were placed in 5 x 8 mm tin cups (Elementar, Hanau, Germany), compressed to a small sphere and analyzed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes at the Laboratory of Stable Isotope Ecology in Tropical Ecosystems, at the University of Miami.

There are higher ratios of ^{13}C to ^{12}C and ^{15}N to ^{14}N in the sea urchin tissues compared to those of their algae food resources, what is referred as Trophic Enrichment Factors (TEFs) (Parnell *et al.* 2010). The TEFs used for the mixture (sea urchin) in all graphs was 2.2 ‰ for $\delta^{15}N$, and 1.0 ‰ for $\delta^{13}C$ (Michener and Schell 1994, Moore and Semmens 2008). Measurements of nitrogen and carbon stable-isotopes abundances were generated in a continuous-flow isotope-ratio mass spectrometer. Results for N and C were expressed as parts per thousand (‰) differences from the corresponding standard (δ) and the standards for $\delta^{13}C$ and $\delta^{15}N$ were Vienna Pee Dee Belemnite (VPDB) and atmospheric N_2 . Carbon and nitrogen samples were analyzed in a dual isotope mode. Standard deviations of $\delta^{13}C$ and $\delta^{15}N$ replicate analyses were estimated once analyses were done. Ratios are expressed as:

$$\delta^{13}\text{C} = \left[\left(\frac{\left(\frac{^{13}\text{C}}{^{12}\text{C}} \right)_{\text{Sample}}}{\left(\frac{^{13}\text{C}}{^{12}\text{C}} \right)_{\text{Standard}}} \right) - 1 \right] \times 1000 (\text{‰})$$

and

$$\delta^{15}\text{N} = \left[\left(\frac{\left(\frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{Sample}}}{\left(\frac{^{15}\text{N}}{^{14}\text{N}} \right)_{\text{Standard}}} \right) - 1 \right] \times 1000 (\text{‰})$$

In addition, we calculated the trophic level (TL) for every individual of *Diadema* in each locality, using the equation of Hobson and Welch (1992), where Nb is the average $\delta^{15}\text{N}$ of the producers (Nb) in every locality, N is the $\delta^{15}\text{N}$ signal of each sea urchin, and TE is the given value for the trophic enrichment factor for ^{15}N (TE), in this case we assumed 2.2 ‰. The Nb was calculated based in the simple unweighted averages (where each algal species has the same contribution to the mean, regardless of its abundance).

$$\text{TF} = \frac{1 + (\text{N} - \text{Nb})}{\text{TE}}$$

Analysis of data- We fitted 2-way ANOVA test to compare the effects of time and site in: total biomass, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for the macroalgal community, and the isotopic signal of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and TEFs of *D. antillarum* and its potential resources among the four sites and two sampling periods. Models incorporated time as fixed factor and site as a random one. A power transformation was used for reaching normality and homogeneity of variance (Box and Cox 1964), where results suggested the square root for biomass, while $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ did not require transformation. When significant differences were detected, we performed a *post-hoc* pairwise comparison of means using Tuckey-HSD test. Prior to

statistical analyses, homogeneity of variances and normality of the variables were checked using Cochran's and Kolmogorov-Smirnov tests, respectively. Thus, we checked the homogeneity of variances and normality of the residuals after the analyses (Zar 2010). No mixing models were run because our data did not fulfill the premises according to Phillips and Gregg (2003). Statistical analyses were performed in the free-license statistical program R-3.0.1 with a $p < 0.05$ (R Core Team 2013).

RESULTS

Thirty nine species of algae were found at the four sites. Fleshy macroalgae represented 76.92 %, while coralline algae represented 23.08 % of the species. The main contributors to biomass were the genera *Halimeda*, *Amphiroa*, and *Galaxaura* in February. *Halimeda* sp. dominated during October, with the other macroalgae remaining below 10% except *Galaxaura* sp. (Fig. 2). Total average biomass fluctuated between $17.45 \pm 23.67 \text{ g.m}^{-2}$ ($\text{mean} \pm \text{S.D.}$) during February, and $26.51 \pm 51.29 \text{ g.m}^{-2}$ on October. The lowest total biomass was recorded in TMD [$8.32 \pm 4.36 \text{ g.m}^{-2}$], while the highest value was reported in LQY [$20.68 \pm 36.49 \text{ g.m}^{-2}$] during October. No differences in total biomass were detected among sites ($\text{df} = 3$, $F = 1.002$, $p = 0.405$), neither between both periods ($\text{df} = 1$, $F = 1.092$, $p = 0.304$).

Macroalgae $\delta^{15}\text{N}$ signals fluctuated from 0.2 to 4.6 ‰. The sites CGD and LQY displayed the highest mean values of $\delta^{15}\text{N}$. From February to October, an increase in the average of $\delta^{15}\text{N}$ was, except in TMD (Tab. 1). Carbon isotope signal ($\delta^{13}\text{C}$) fluctuated between -20.1 to -6.4 during February, with an overall season average of -11.9 ± 3.7 ‰, with CGD and LQY displaying the most negative values (Fig. 3). During October sites

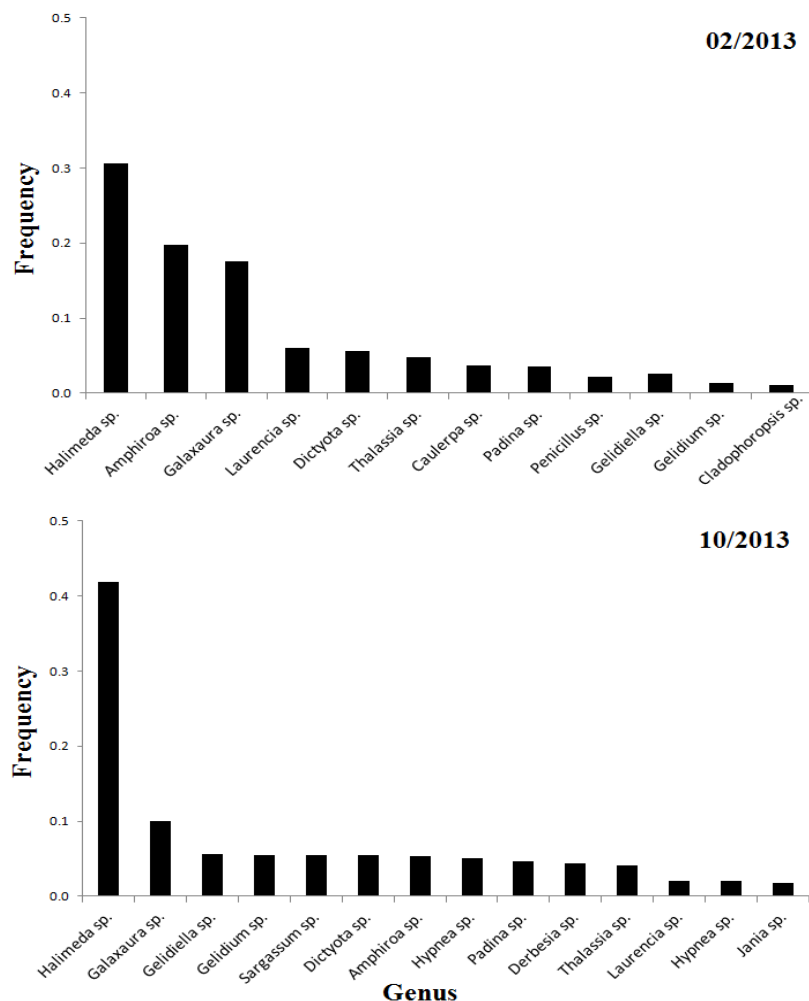


Figure 2 Relative abundance (in terms of biomass) of the main contributor genera of macroalgae for both sampling periods.

exhibited similar values, with $\delta^{13}\text{C}$ fluctuating between -18.7 and -13.8 ‰. We did not find spatial or temporal differences in terms of $\delta^{13}\text{C}$. In contrast, significant spatial and temporal variability was observed with respect to $\delta^{15}\text{N}$ (Tab. 1). A posteriori analysis showed that the main differences among sites was between MLN and CGD ($p= 0.0007$), MLN and LQY ($p= 0.003$), and TMD and CGD ($p= 0.032$) during February; whereas MLN and LQY were the only two sites that differed significantly in October ($p= 0.023$).

Table 1. Mean average \pm SD of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for the macroalgal community in the four sites (CGD, LQY, TMD and MLN) and two sampling dates (Feb and Oct, 2013). Results of 2-way ANOVA on the isotopic signals of carbon and nitrogen of all primary producers testing for differences between sites and sampling periods and their interaction are also presented. Asterisks indicate significance, SS is the sum of square and MS is the mean of square.

	$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)		
	February	October		February	October	
CGD	-12.9 ± 3.86	-13.9 ± 4.09		2.3 ± 1.86	3.0 ± 1.01	
LQY	-10.7 ± 4.50	-15.4 ± 5.76		2.1 ± 1.1	3.0 ± 0.76	
TMD	-12.6 ± 3.66	-11.7 ± 2.92		1.8 ± 0.87	1.6 ± 0.61	
MLN	-12.1 ± 3.59	-11.9 ± 3.87		0.9 ± 1.49	1.3 ± 0.84	
	df	F	p	df	F	p
site	3	0.776	0.509	3	16.69	0.0001*
time	1	38.31	0.144	1	6.09	0.0001*
sxt	3	46.24	0.055	3	1.37	0.387
		SS	MS		SS	MS
error	113	2000.301	17.701	113	151.59	1.341

The isotopic signature of *D. antillarum* during February varied from 6.69 ± 1.02 to 8.02 ± 0.14 ‰ for $\delta^{15}\text{N}$; while $\delta^{13}\text{C}$ fluctuated between -12.4 ± 1.05 and -9.21 ± 0.43 ‰.

Nitrogen isotopic signal varied from 6.27 ± 0.18 to 8.21 ± 0.36 ‰, while $\delta^{13}\text{C}$ fluctuated between -13.84 ± 0.90 ‰ to -9.25 ± 0.61 ‰ on October (Fig. 4). The analysis of variance detected differences for both isotopes among sites, but not between seasons. A posteriori analysis found that LQY differed with respect to TMD ($p=0.007$), and MLN ($p=0.009$), but not with CGD ($p=0.998$) in terms of $\delta^{15}\text{N}$. Results of $\delta^{13}\text{C}$ showed similarities only between CGD and LQY ($p=0.998$) (Tab. 2).

Trophic enrichment factor for all sites Northeastern coast of Puerto Rico was 2.69 ± 0.15 in February, and 2.94 ± 0.04 in October. The highest TEFs were reported in CGD and MLN during October, while the lowest TEF was recorded in CGD during February (Fig. 5). Remarkable differences among sites and between times were found (Tab. 2).

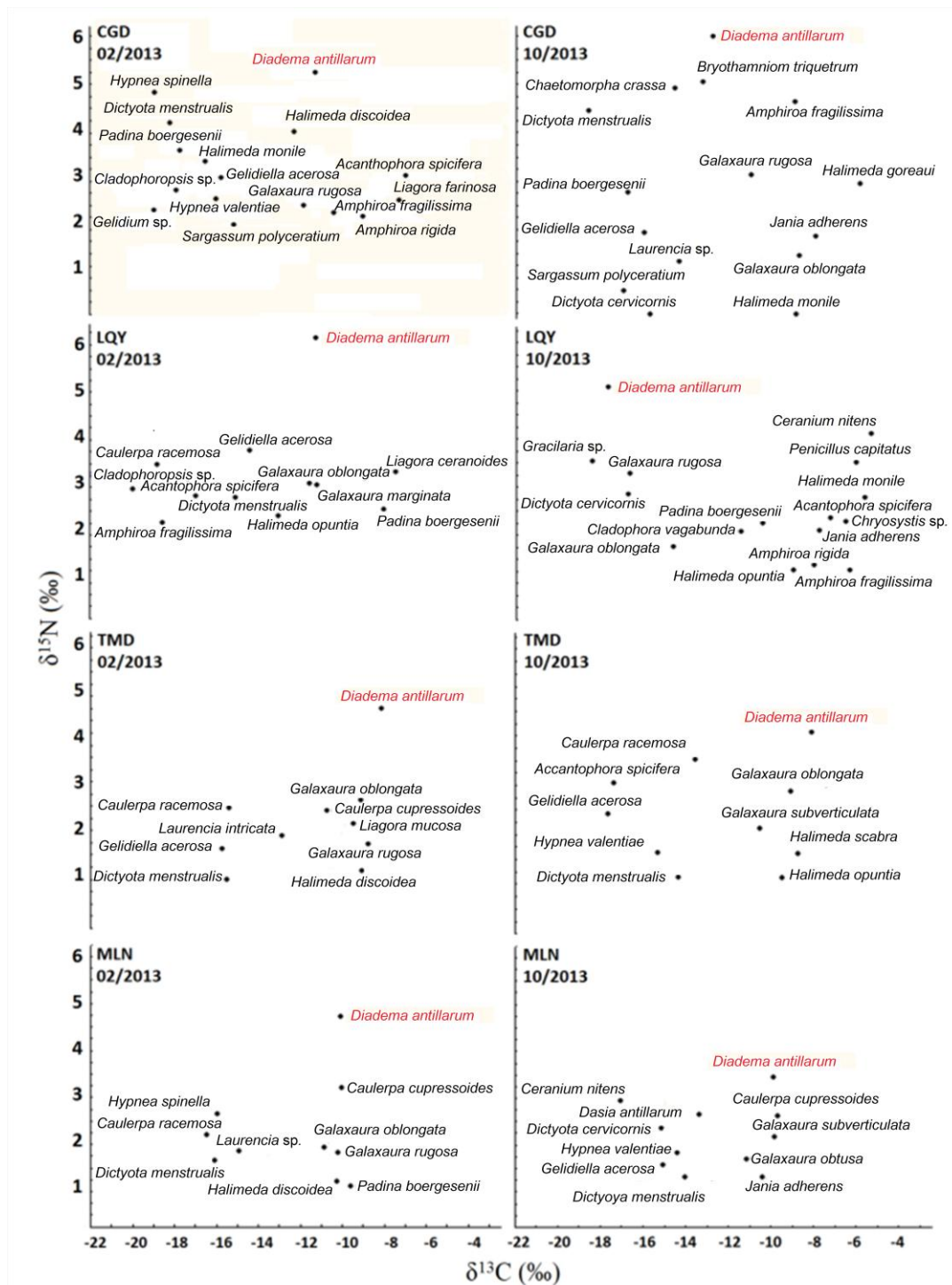


Figure 3 Trophic general signatures of the sea urchin *Diadema antillarum*, and its potential food resources for the four study sites (CGD, LQY, TMD and MLN) and the two sampling periods (Feb 2013 and Oct 2013). Trophic enrichment factors used for the mixture (sea urchin) in all graphs were 2.2 ‰ for $\delta^{15}\text{N}$, and 1.0 ‰ for $\delta^{13}\text{C}$. We include in the graph only the macroalgae with the isotopic signal closer to the consumer (red color).

Table 2. Two-way analysis of variance of the effects of time and site, on the isotopic signatures of carbon, nitrogen, and the trophic enrichment factors (TEFs) of the sea urchin *D. antillarum*; asterisks indicate significance, SS is the sum of square and MS is the mean of square, SS is the sum of square, and MS is the mean of square.

	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$			TEFs		
	df	F	p	df	F	p	df	F	p
time (t)	1	0.460	0.502	1	0.921	0.3446	1	16.67	< 0.0001*
site (s)	3	32.95	0.0001*	3	76.67	0.0001*	3	10.27	0.0003*
s x t	3	2.831	0.0547	3	5.850	0.0027*	3	15.08	< 0.0001*
		SS	MS		SS	MS		SS	MS
error	31	5.495	0.177	31	10.542	0.340	31	1.095	0.035

A posteriori analysis found that all sites exhibited temporal differences within themselves (Tuckey-HSD test, $p < 0.05$). Thus, differences in TEFs exhibited significant spatial differences within the same time period. For instance, CGD was different respect to all sites, except with LQY in February; whereas in October, TMD exhibited differences respect to the other three sites ($p < 0.05$). Mixing models were not performed because for all sites and periods of time, all isotopic signals of algae in the Y axis, after subtracting the trophic enrichment factors, remained below the *Diadema* isotopic signal of $\delta^{15}\text{N}$, and this spatial configuration of food sources with respect to the mixture (herbivore) does not allow running mixing models (see Phillips and Gregg 2003).

DISCUSSION

Human development has impacted the coastal zones all over the world through the use of fertilizers, deforestation, and land erosion processes, increasing terrestrial nutrients inputs into marine ecosystems (Cloern 2001). Terrestrial inputs in marine ecosystems are usually responsible for differences in the $\delta^{15}\text{N}$ signal of marine organisms (Gacía *et al.* 2002), which is related to the local land-use history (Clark and Wilcock 2000). Marine

communities affected by sewage tend to display higher signals of $\delta^{15}\text{N}$ than pristine environments (Kendall and McDonnell 1998). Indeed, elevated values of $\delta^{15}\text{N}$ in marine macroalgae tissues have been linked with NO_3 enrichment, a molecule very common in urban discharges (Heaton 1986). Some studies have reported values between 3-8 ‰ as a signal of eutrophication (Lapointe *et al.* 2004, Aguilar *et al.* 2008).

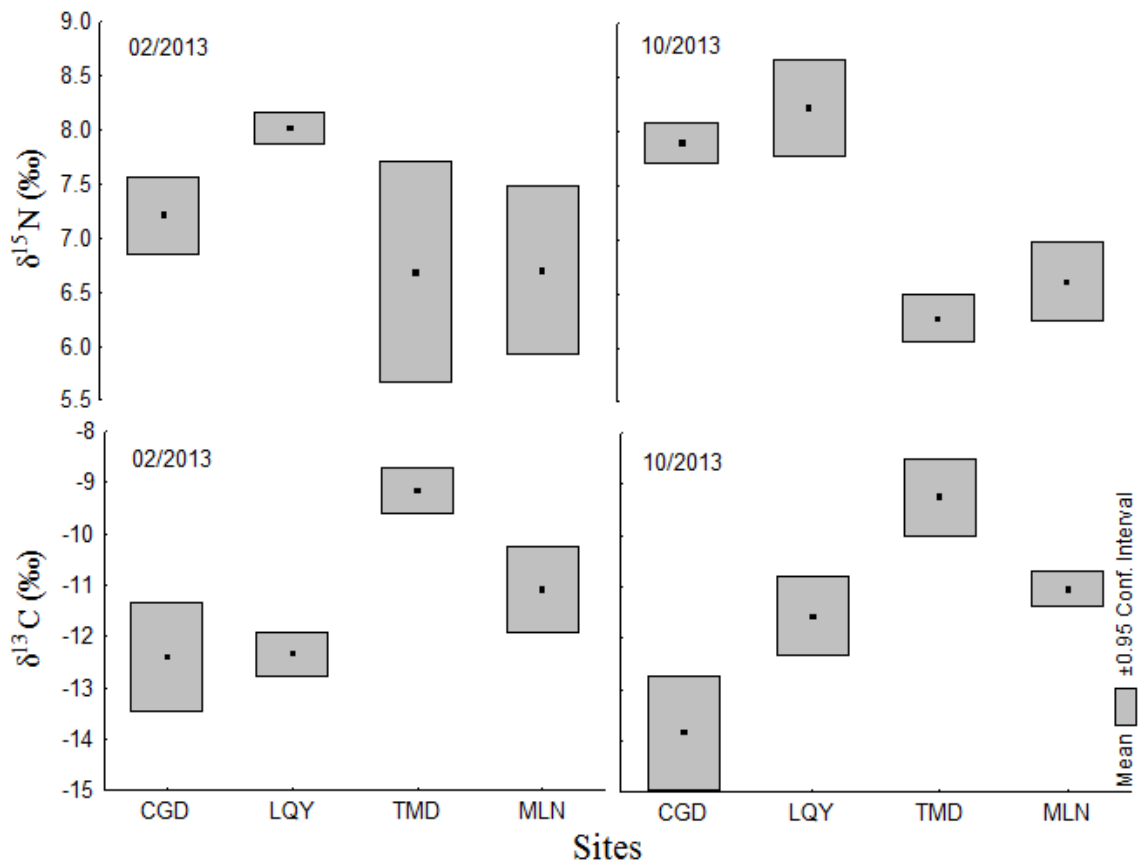


Figure 4 Stable isotope signals of Nitrogen ($\delta^{15}\text{N}$) and Carbon ($\delta^{13}\text{C}$) of the sea urchin *Diadema antillarum* in four sites of Puerto Rico and two sampling dates. Gray boxes represent the 95% of confidence interval of the mean.

In this study, we found that $\delta^{15}\text{N}$ signal from macroalgae never reached 3 ‰ in TMD and MLN, but some species displayed values over this threshold in CGD and LQY. Previous studies conducted in Mexico and Puerto Rico exhibited lower values of $\delta^{15}\text{N}$ for some

common genera such as *Penicillus*, *Halimeda*, and *Galaxaura* sp. (France *et al.* 1998, Lacey 2012). The same pattern was observed in the genera *Halimeda*, *Jania*, and *Padina* in a study conducted in Hawaii. The author found that these species displayed higher values of $\delta^{15}\text{N}$ (Derse 2006). Spatial difference in $\delta^{15}\text{N}$ among sites observed in this study (Fig. 3) may be explained by the influence of terrestrial inputs derived from river discharges. The source of nitrogen inputs in CGD seems to be the mangrove system channel located 270 meters from the study collection site. The LQY site may be influenced by the discharge of the Pitahaya and Mameyes rivers, whereas TMD and MLN do not have any river discharge influence (U.S.G.S. 1996).

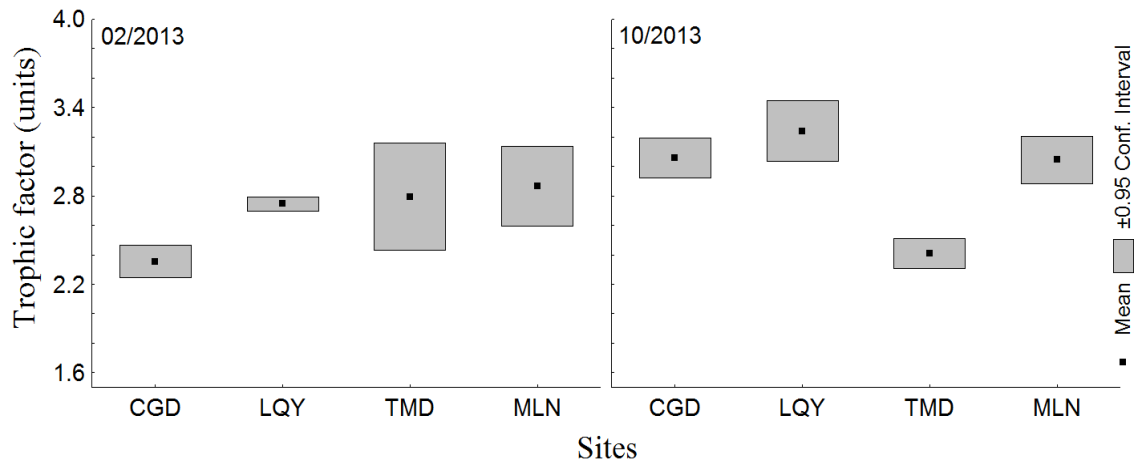


Figure 5 Average trophic factor enrichment of the echinoid *Diadema antillarum* in four sites of Puerto Rico and two sampling dates. Gray boxes represent the 95% of confidence interval of the mean.

The isotopic signals of nitrogen and carbon in consumers are influenced by local availability of food resources (Phillips *et al.* 2014). *D. antillarum* was characterized isotopically in Bahamas (Keegan and De Niro 1988), while a recent study conducted in the Canary Islands with *Diadema africanum* exhibited spatial, and also seasonal changes in the isotopic signals of *Diadema* (Cabanillas-Terán 2009). Seasonal differences could

be in consonance with annual changes in temperature. Seasonal fluctuations in temperature cause structural changes in the seaweeds communities (Lirman and Biber 2005), that has an effect in the availability of food resources, and the contribution of each source to the mixture. Nevertheless, seasonal differences are stronger in the Canary Islands, whereas Puerto Rico exhibits lower fluctuations in annual temperature (NOAA Coral Reef Watch 2000, Hernández *et al.* 2011). This would tend to keep the stability of algal sources throughout the year.

A theoretical premise of mixing models is that there is no solution when the consumer remains outside a hypothetical polygon bounded by all food resources (Phillips *et al.* 2014). Mixing models on *Diadema africanum* found that this echinoid assimilates different algae genera depending on spatial and temporal availability (Cabanillas-Terán 2009), and the existence of these polygons support the assimilative herbivory on *D. africanum* in the Eastern Atlantic. In this study however, all potential food resources (seaweeds) remained under the $\delta^{15}\text{N}$ signal of the consumer, suggesting the existence of other non-seaweeds/plant resources in the items ingested and assimilated by *D. antillarum*. The lack of congruent polygons formation avoid running mixing models, but also indicate that *D. antillarum* assimilative process is more complex than that of *D. africanum*. Nevertheless, the signal of $\delta^{13}\text{C}$ in the genera *Galaxaura* and *Halimeda* were closer to the isotopic signal of carbon of *D. antillarum* (Figs. 2 & 3). These two genera of macroalgae were the most important contributors in biomass, which suggests the importance of *Galaxaura* and *Halimeda* as important sources of carbon for this echinoid. It also indicates that *D. antillarum* assimilates the food resources according to their availability in the ecosystem.

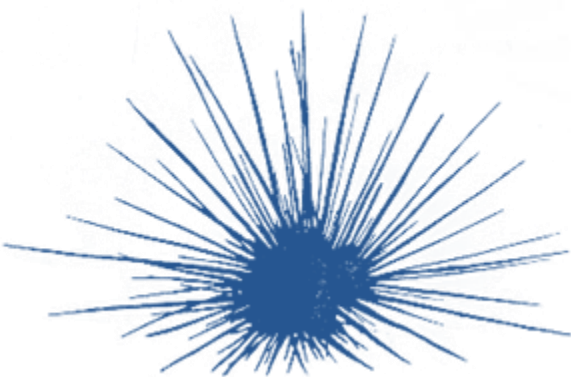
In a recent study, authors found similar values of $\delta^{15}\text{N}$ in other omnivores echinoderms (Hobson *et al.* 2002). For instance, the echinoid *Strongylocentrotus pallidus* and the brittle star *Ophiophthalmus sarsi* exhibited $8.3 \pm 0.5 \text{ ‰}$ and $9.7 \pm 0.3 \text{ ‰}$ respectively (Hobson *et al.* 2002). A similar result was reported by Cocheret de la Morinière *et al.* (2003), who measured the isotopic signal of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for different taxonomic groups in the Caribbean. They found that decapods, fire-worms, and amphipods isotopic signals occupied a higher position with respect to the omnivore sea urchin *Tripneustes ventricosus*. Even though authors did not run mixing models, the isotopic nitrogen signal of *T. ventricosus* was similar to the position of *D. antillarum* found in this study. This similarity may suggest a similar trophic position between both species (Vanderklift *et al.* 2006, Phillips *et al.* 2014).

Even though *D. antillarum* has been reported as feeding primarily on algae (Lewis 1964), it also ingests flowering plants and detritus (Randall *et al.* 1964). In addition, sponges, hydroids, bryozoans, nematodes, rotifers, gastropods, bivalves, and copepods were the most common groups of invertebrates found in gut studies on *Diadema* (Hernández *et al.* 2006). Herrera-López *et al.* (2003) found that between 24 to 33 % of the digestive content of *D. africanum* were invertebrates. Coral fragments and zoanthids have also been reported in stomach content of *D. antillarum* (Karlson 1983). The small invertebrates that inhabit macroalgae clumps (*pers. obs.*) may be consumed by *D. antillarum* while ingesting seaweeds because of their relative small sizes. In addition, organisms without hard structural elements, such as ascidians and some sponges, might leave no traces in the digestive system after a short period of time. Microbial activity may also lead to changes in the isotopic composition of the ingested material (Vanderklift and

Ponsard 2003, Vanderklift *et al.* 2006). Another potential food source of nitrogen to be considered in further analysis is the thin layer over the substrate (biofilm). The content of $\delta^{15}\text{N}$ accumulated in the biofilm, more in areas impacted by human discharges than pristine environments (Kendall and McDonnell 1998), could be incorporated to the digestive system when *D. antillarum* scrape the substrate. However, the $\delta^{15}\text{N}$ of biofilm is consistently lower than that of macroalgae in most published studies. So, the intake of biofilm would not explain the higher $\delta^{15}\text{N}$ signature of *Diadema*. Then, the most parsimonious explanation is the intake of invertebrates.

This potential explanation is supported for the high TEFs values found for *D. antillarum*, that also goes against the classification of the species as herbivore. TEFs higher than 2.35 ± 0.11 and up to 3.24 ± 0.17 agree with a recent study conducted with the sea urchin *Arbacia lixula*. This Mediterranean species was being considered to feed mainly on encrusting coralline algae, but a recent study found TEFs closer to carnivores (Wangensteen *et al.* 2011). These results goes against the widespread believe of an herbivorous diet for *A. lixula*, and now for *D. antillarum*. Therefore, the presence of small invertebrates during gut content analyses, including coral fragments in *D. antillarum* (Rylaarsdam 1983, Herrera-López *et al.* 2003) may not be an accidental ingestion after all. The lack of congruent polygons found in this study, and high TEFs reinforce the importance of invertebrates as potential resources of nitrogen. We conclude that our results support that *D. antillarum* must be considered an omnivore in terms of assimilation. Further studies, where small invertebrates samples were included, may help to quantify the contribution of the whole food sources of *D. antillarum*.

Evaluation of three tagging methods in the
sea urchin *Diadema antillarum*



ABSTRACT

Multiple tagging devices have been developed for long-term studies and estimating demographic parameters in sea urchins. In this study, we evaluated the use of passive integrated transponders (PIT-tag), and two types of nylon tags (T-bar and S-tag) in the sea urchin *Diadema antillarum* by measuring retention rate and apparent survival. The PIT-tags exhibited the highest retention, followed by T-bars, and lastly the S-tags. Differences in recapture were detected among the three types of tags ($H= 6.99$, $p= 0.030$). A posteriori pairwise comparison test found significant differences between PIT-tags and each of the other two types ($p < 0.05$), whereas T-bar and S-tag did not exhibit remarkable differences between them ($p > 0.05$). The semi-captivity experiment exhibited similar results to the field experiment in terms of retention. This experiment also found higher mortality with T-bars. Differences between previous studies conducted under controlled conditions and experiments carried out in the field reflect high variability and the necessity of testing tagging procedures under both settings. The S-tag induced high spine autotomy and low retention; whereas the T-bar demonstrated low retention and low survival. Although the retention rate of PIT-tags was significantly higher than the other two, retention rates were still too low for practical utility in long-term field experiments. In conclusion, the present study did not support the use of any of these tags for long-term studies in *D. antillarum* due to their low retention.

INTRODUCTION

Long-term studies have become an important tool for the estimation of survival, recruitment, and growth rates needed for the parameterization of demographic models (Pollock *et al.* 1990, Pradel 1996, Mowat and Strobeck 2000, Caswell 2001, Lauzon-Guay and Scheibling 2008) to better understand the population dynamics of marine species. The use of external and internal devices have been very useful in identifying invertebrates, fish, birds, reptiles, and mammals in the field and in controlled environments (Prentice *et al.* 1990, Schooley *et al.* 1993, Steyernark *et al.* 1996, Duggan and Miller 2001, Nicolaus *et al.* 2008, Rodríguez-Barreras *et al.* 2014b). These types of studies need high tag retention rates and neutral effects on survival, growth, movement, etc. (Williams *et al.* 2002, Amstrup *et al.* 2006).

Multiple tagging techniques have been developed for sea urchins, such as the insertion of metallic labeled bars into the coelomic cavity, tetracycline marks, plastic tags attached to the spines, painted madreporite, or anchored labels attached to the animal's test (Walker 1981, Ebert and Russel 1992, James 2000, Agatsuma *et al.* 2000, Duggan and Miller 2001; Cipriano *et al.* 2014). Recently, the Passive Integrated Transponder (PIT) tag has become one of the most common internal devices used for accurate and individual identification of sea urchins (Hagen 1996, Hazan *et al.* 2014). Laboratory and field studies with sea urchins have recorded more than 90% retention with PIT tags and low mortality rates (Sonnenholzner *et al.* 2010, Rodríguez-Barreras and Sonnenholzner 2015).

The sea urchin *Diadema antillarum* (Philippi, 1845) is one of the most common species in the Western Atlantic. The species suffered a population collapse in the early 1980's caused by an unknown pathogen (Lessios 2013). Three decades later, *D. antillarum* has partially recovered but the process has not been homogeneous and densities remain far from pre-mortality levels throughout the Gulf of Mexico and the Caribbean (Lessios 2013, Rodríguez-Barreras *et al.* 2014a). The development of demographic models for a better understanding of *Diadema* population dynamics is critical to informing proper management and conservation decisions that promote re-establishment of current populations. Surgical tubing attached to the spines, hooks fastened to a fishing line inserted in the aboral membrane, and nylon T-bars crossing the test have all been used in movement and predation studies on the genus *Diadema* (Carpenter 1984, Tuya *et al.* 2003, Clemente *et al.* 2007), but there is no information related with the use of PIT-tags in the genus.

The development of suitable and effective tagging methods for *D. antillarum* constitutes a serious challenge for the development of long-term studies that include capture-mark-recapture techniques. The main goal of this study was to determine the most effective tagging method for *D. antillarum* using three different types of tags. We hypothesized that tagging *D. antillarum* with PIT-tags will result in higher retention and lower mortality rates than with external nylon tags (T-bar / Snap lock tags).

METHODS

The study was conducted in four fringing reefs, located in northeastern Puerto Rico and Culebra Island: Cerro Gordo (CGD: 18°16'51.40"N, 65°17'12.21"W), Luquillo (LQY:

18°23'18.46"N, 65°43'5.52"W), Tamarindo Bay (TMD: 18°18'55.29"N, 65°19'5.82"W), Melones (MLN: 18°18'15.70"N, 65°18'42.27"W), and Key Largo (CLG: 18°18'31.29"N, 65°34'48.48"W), see Fig. 1. Study area descriptions appear in Rodríguez-Barreras *et al.*, (2014a). The experiment was carried out between October and December of 2012; during which field and enclosed experiments lasted 1 month each.

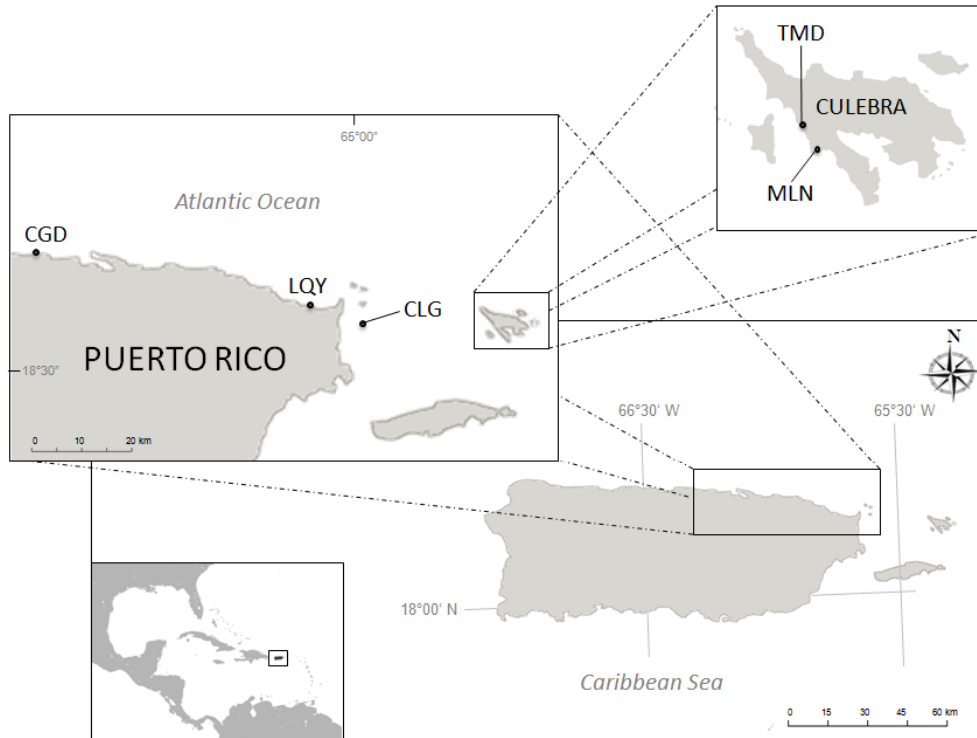


Figure 1 Map showing the five study sites: Cerro Gordo (CGD), Luquillo (LQY), Key Largo (CLG), Tamarindo Bay (TMD) and Melones (MLN).

Experimental setup: We established 3 circular plots of 5 m diameters, each separated by 20 m, with the center of each circle marked with iron bars to avoid using the same sea urchin in more than one treatment. The sea urchin *D. antillarum* has a well established “homing behavior”, where individuals usually do not displace more than 4 m from the site used as refuge (Carpenter 1984). A buffer zone of an additional 5 m radius from the border of the plots was established to check for any potential displacement of individuals

outside of the study plots. We tagged only individuals of *D. antillarum* greater than 40.0 mm of horizontal test diameter. We used PIT-tags, and two external tags: tag fasteners inserted through a hole in the test (T-bar), and snap lock pins attached to one of the urchin's spines (S-tag) (Fig. 2). For field experiments, each type of tag was tested in different months. We did not tag the same sea urchin with more than one type of tags.

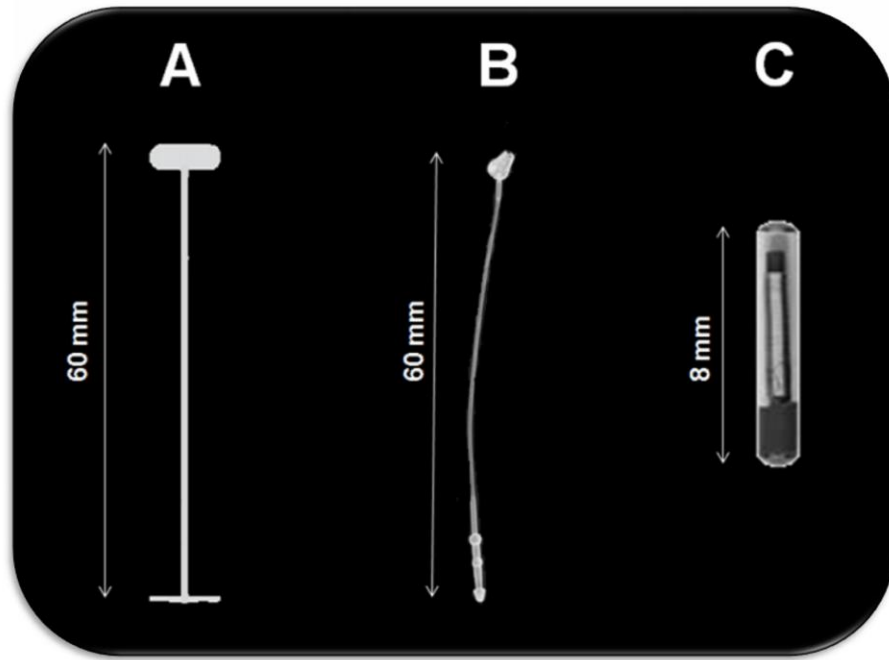


Figure 2 Three types of tags used in the sea urchin *Diadema antillarum* were, A: T-bar, B: S-tag, and C: PIT-tag.

Tags were tested in the field under two conditions: enclosed and free ranging. We selected 20 sea urchins for each trial at each location. PIT-tags were tested at CGD, LQY, TMD, and MLN; while T-bars and S-tags were tested in all locations except at CGD. The site LQY was the only one checked weekly during the experiments. With the resulting data, we calculated the percent of recapture for each treatment, which is the number of tagged sea urchins recaptured after a month, and would be equivalent to retention under

field conditions. All individuals were tagged *in situ* and immediately liberated after tagging.

Another experiment was conducted to measure the effect of tags on mortality and retention under semi-captivity conditions. A group of 10 sea urchins per treatment (one control and three tag treatments) were placed inside bottomless metallic cages (2 m length x 1 m height x 1 m wide) during one month in CLG with no replicates. Sea urchins were assigned randomly to treatments and each cage represented a treatment to allow correct individual/treatment identification.

T-shape fasteners tag (T-bar): Individuals were tagged using a Fine Tagging Gun. Light pressure was applied in the inter-ambulacral region until the test was perforated and a hole of 1.5 mm was made. Once the needle was inside the coelomic cavity, the tagging gun was engaged and the tag attached against the inside wall of the test. The distal part of the tag outside the urchin was labeled with a number code (Fig. 2a).

Snap lock pin (S-tag): The S-tag has a structure composed of three proximal teeth with a cavity in the middle of one side and a distal 40 mm tail (Fig. 2b). We added a label in the distal side for identification purposes. We attached the tag to a spine close to the peristome membrane for maximum visibility (Fig. 3), using a pipette to push the proximal side of the tag through the spine close to its base. Once the tag was in place, the pipette was withdrawn, and the tag fixed to the spine by adding a droplet of a non-toxic Ecotech Marine Coral Glue (www.ecotechmarine.com).

Passive Integrated Transponders (PIT-tag): Sea urchins were tagged *in situ* with a 8 mm PIT-bar (HDX-134.2 kHz, Fig. 2 C) and their test diameters were measured with a caliper

(error ± 0.5 mm). The PIT-tag was inserted through the peristome membrane and into the coelom cavity with a plastic injector and a 2.15 mm diameter needle. Sites were visited one month later and sea urchins identified with a handheld portable PIT-tag reader.

Statistical analysis- We ran a non-parametric analysis of variance (Kruskal-Wallis) to test differences in percent of recapture among treatments (Zar 2010). We performed a pairwise comparison (Mann-Whitney U-test) to check which pairs of tags were significantly different from each other. This analysis was also performed to test for differences in mortality and retention among treatments. Statistical analyses were performed in the free-license statistical program R version 3.0.1 with an alpha value of 0.05 (R Core Team 2013).

RESULTS

Open-field experiment: Recapture rates among all three tagging methods were low, with significant differences in recapture detected among tagging methods (Kruskal–Wallis test, $df = 2$, $H = 6.99$, $P = 0.030$). The PIT-tag exhibited the highest retention rate, followed by T-bars, and lastly the S-tags (Fig. 3). A posteriori pairwise comparison test found significant differences between PIT-tags and each of the other two types ($p < 0.05$), whereas T-bar and S-tag did not exhibit remarkable differences between them ($p > 0.05$). The PIT-tag recaptures averaged 32.5 ± 22.18 %, and exhibited high variability among sites. Recaptures ranged from 10% in LQY to 60 % in MLN. In LQY, where individuals were followed weekly, the greatest loss of PIT-tags occurred during the first week, with 35% of the tags lost (Fig. 4). With the T-bar, we obtained an average recapture of 1.67 ± 2.89 %, with no recaptures in TMD and MLN, and a maximum of 5 % in LQY (Fig 3).

Weekly sampling showed a constant decline in retention. Recaptures decreased 50% after the first week, and maintained this declining trend until the last week, in which no recaptures were identified (Fig. 4). Some carcasses found in LQY, were recognized for the presence of the drilled holes (Fig. 5A).

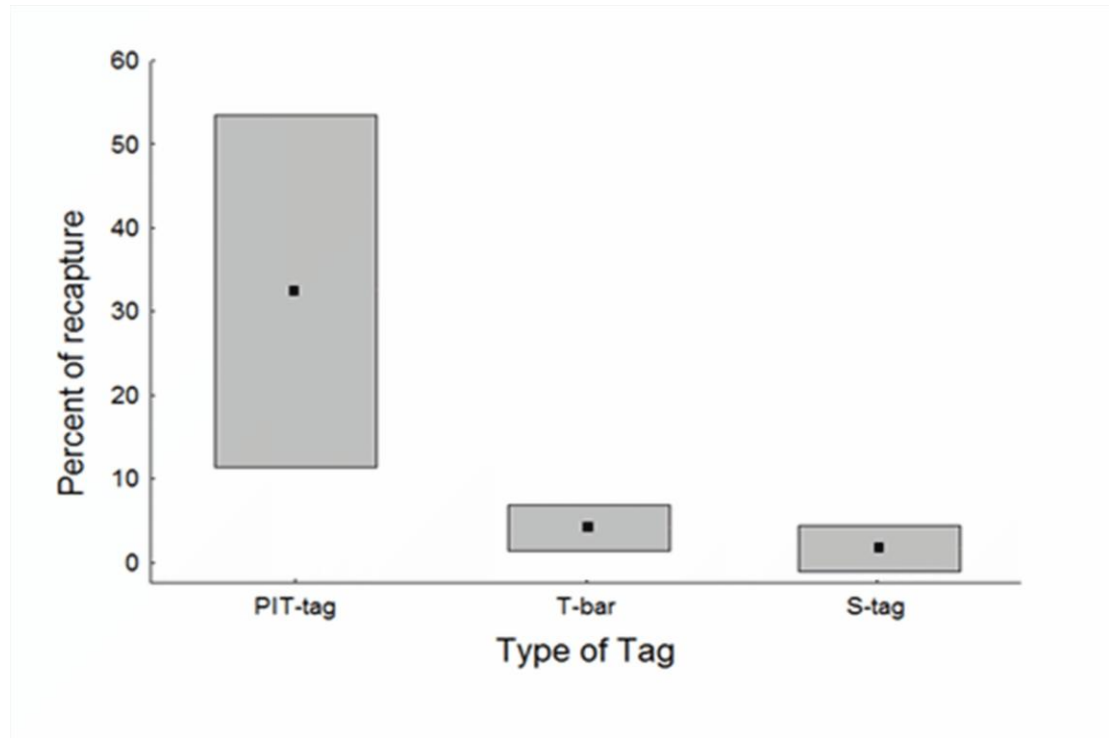


Figure 3 Overall recaptures (mean \pm SD) of three different tagging methods for the sea urchin *Diadema antillarum* after one month at four sites for PIT-tag, and three sites for S-tag and T-bar, all in the northeastern coast of the Puerto Rico Archipelago.

S-tag recaptures were 3.33 ± 2.89 %, and ranged from no recaptures in TMD, to a maximum of 5% in LQY and TMD (Fig. 3). Particularly in LQY, recaptures dropped 85% at the end of the first week, and only 5% retained the S-tag after a month (Fig. 4). Spine autotomy was detected in S-tagged individuals. Some entire spines with the S-tag still attached were found in the field, showing evidence of high rates of autotomy. Some echinoids were easily recognized for the absence of one large dorsal spine and the

presence of a naked tubercle (not covered with epidermis), which indicates a recent autotomy event. Indeed, we recorded 10% of autotomy with S-tags during the first hour after the procedure (Fig. 5 C & D). This tagging procedure was time consuming due to the excessive spine and pedicellariae movement, a natural response when a sea urchin is manipulated.

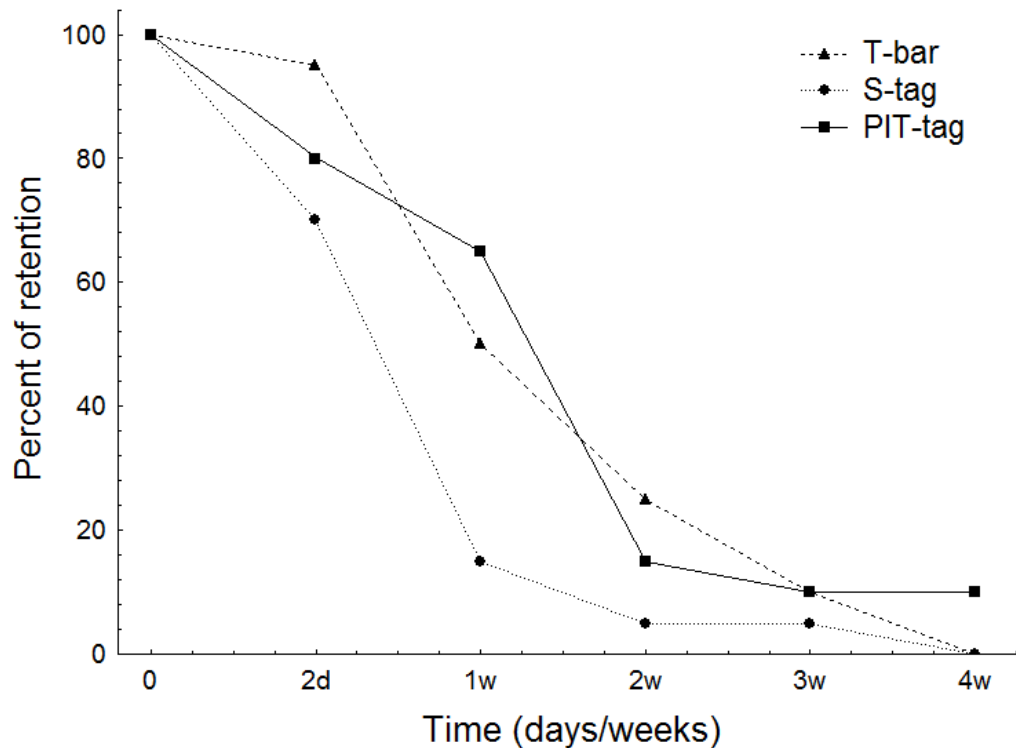


Figure 4 Recapture rate (without replicates) of three different tags for the sea urchin *Diadema antillarum* at Luquillo Beach (LQY).

Semi-captivity experiment: In the experiment where sea urchins were tagged and placed into metallic cages for one month, CLG individuals exhibited low tag retention for all three tags. Echinoids exhibited 30 % of retention and 20 % of mortality with PIT-tags. Individuals with no signal were dissected to eliminate a potential reading error, and no PIT-tag was found in the coelomic cavity. T-bar tagged sea urchins exhibited 40%

mortality after a month, and surviving individuals had not tag (i.e. 0% retention). Two live individuals showed the presence of a circular layer of fine sediment covering the affected area (Fig. 5A); whereas the other three individuals showed evidence of the drilled area covered by new epidermis. The tests belonging to dead individuals exhibited evidence of necrosis in the drilled area (Fig. 5B). No mortality, but zero retention rates were recorded for S-tags after a month. Control sea urchins exhibited no mortality either.

DISCUSSION

The major goal of this study was to provide information about the effectiveness of three different tags in the sea urchin *D. antillarum*, both under semi-captivity and field conditions. Several studies have previously reported high retention rate and low mortality in laboratory trials with PIT-tags in echinoids (Hagen 1996, Cipriano *et al.* 2014, Hazan *et al.* 2014). High retention and survivorship are required for a successful and accurate long-term capture-mark-recapture study aimed at estimating vital rates (Williams *et al.* 2002, Amstrup *et al.* 2006). For instance, a study conducted with the echinoid *Strongylocentrotus purpuratus* recorded 100% retention rate (Sonnerholzner *et al.* 2010), while the same technique tested in another sea urchin reported more than 90% of retention and no mortality after two months (Hagen 1996). However, another study conducted in *Strongylocentrotus droebachiensis* found low retention and survival under field conditions (Lauzon-Guay and Scheibling 2008). Our results differed from these studies because retention/recaptures with PIT-tags were lower than expected. The highest recapture rate of 60% recorded in one of the three localities cannot be considered suitable for long-term studies.

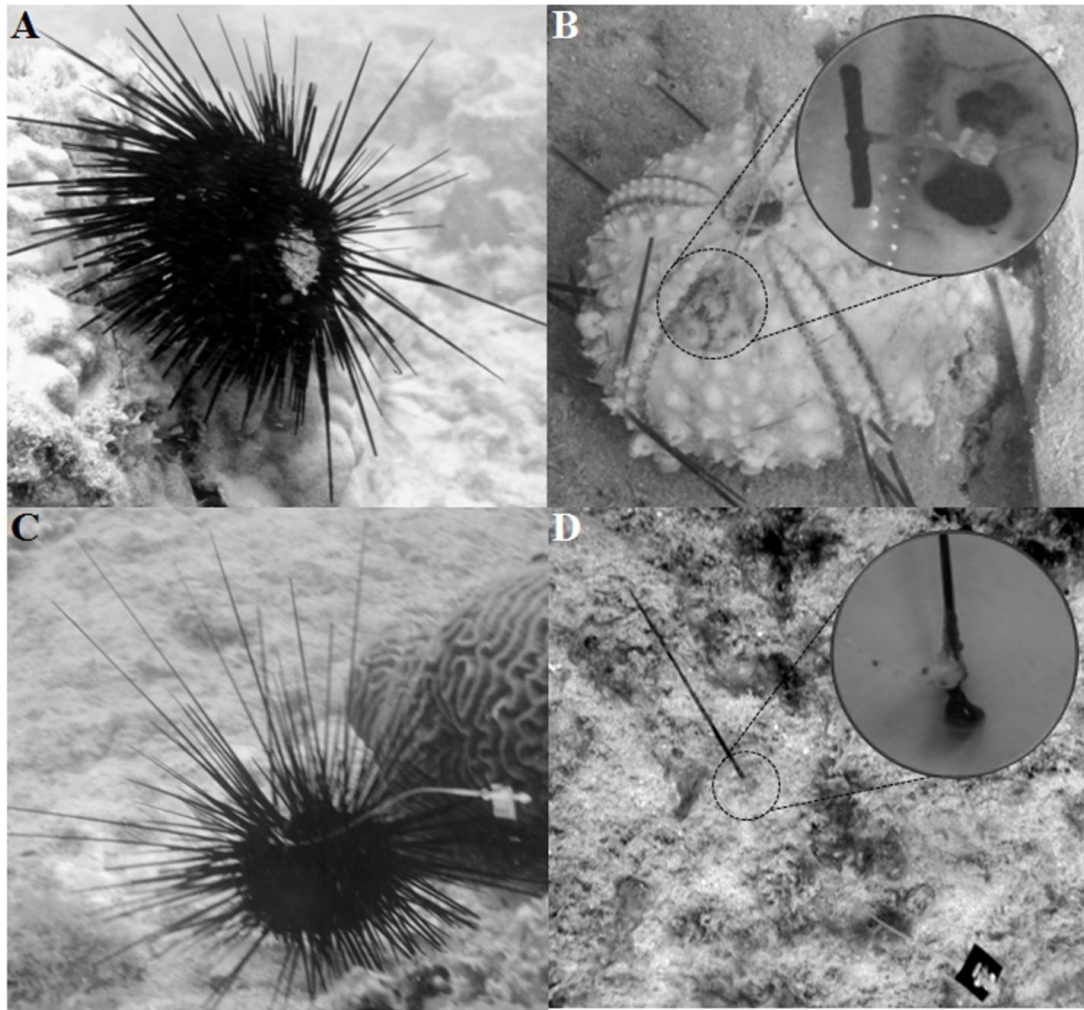


Figure 5 Detail of a live sea urchin *Diadema antillarum* with a damaged area covered with fine sediment after the loss of the T-bar (A). The same species with the T-tag still inserted (B). Notice the internal view of the same test with the base of the T-bar and the drilled area with necrotic signals (black color). Sea urchin carcass with the S-tag attached to the aboral region (C), and D shows evidence of autotomy response to the presence of T-bar.

The majority of studies with PIT-tags in echinoids have been conducted under controlled conditions in laboratories and other marine facilities, but not in the field (Kalvas *et al.* 1998, Palleiro-Nayar *et al.* 2009, Sonnernholzner *et al.* 2010). Differences in retention and mortality rates between field and laboratory experiments suggest that environmental conditions decrease the efficacy of PIT-tags in field studies. However, Cipriano *et al.*

(2014) found high recapture with *Paracentrotus lividus* in the field, which disagrees with our results. Low retention obtained here for PIT-tags demonstrate that field conditions may diminish the effectiveness of tags, lowering retention/recapture rates.

Previous evaluations of T-bars in other echinoids have recorded low mortality rates (Dix 1970, Duggan and Miller 2001). Low survival and low retention obtained here demonstrates the unsuitability of this technique for long term field studies. This invasive device in which the test is drilled may cause internal infection, and induces necrosis around the affected area (Fig. 5B). Indeed, Olson and Newton (1979) reported epithelium growth around the drilled area, but the test did not recalcify properly. Furthermore, substrate complexity may play an important role for external tags. The external T-bar is exposed directly to substrate irregularities, being lost easily in the field, but not in fish tanks where rugosity is not significant. T-bars may have become lodged in the wall of the metallic cages or between rocks, and subsequently lost. The other tested external tag, however, could reduce loss probabilities due to its pointed distal shape (Fig. 2).

This was the first time that S-tags were used in *D. antillarum*. This type of tag is considered the least invasive because it does not require the entrance of any device into the coelomic cavity. Nevertheless, attaching a device to *Diadema* spines is not new. A similar method was used on *D. antillarum* in which numbered surgical tubes were inserted in the spine (Carpenter 1984); whereas we used a toothed structure (Fig. 2) fixed with a non-toxic glue to minimize the pedicellariae removing effect. The sea urchin detected the presence of a non self- object and induced an autotomy response. Carpenter (1984) was able to identify individuals during 6 months and reported an autotomy rate of 10 %. However, we recorded 100 % autotomy and no recaptures after one month. We

speculate that some chemical components of the non-toxic glue have lead to an increase in autotomy or that the length of the tag made its detection easier for *Diadema*, which released the entire spine from the base including the attached tag (Fig. 5D).

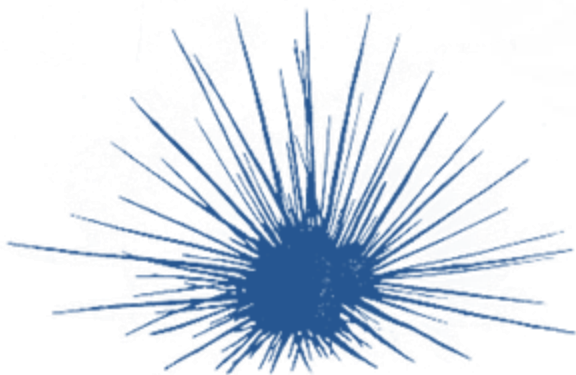
Another negative factor which may negatively influence our results is predation, particularly with T-bars. Mixed groups of the small wrasses *Thalassoma bifasciatum* and *Halichoeres bivittatus* were observed surrounding and aggressively biting urchins recently tagged with a T-bar. Both of these carnivorous fishes have been previously reported as potential predators of *D. antillarum* by Lessios (1988). Moreover, the existence of an injured area (where the T-bars attached to the test) occasionally attracted the polychaete *Hermodice carunculata*. In more than 15 occasions we recorded the presence of this fire worm on recently T-bar tagged *D. antillarum*. This polychaete moved through the spine canopy until it found the hole, and removed living tissue around the drilled zone. On the other hand, we also confirmed the homing behavior of *D. antillarum* proposed by Carpenter (1984). 95% of the tagged sea urchins moved no more than 5 m from the designated circular plots. This result supports the conclusion that low recaptures were not mainly related to movements of *Diadema*, outside the study plots.

Since the PIT-tags have been successfully used in other sea urchin species (e.g. Cipriano *et al.* 2014 in *Paracentrotus lividus*, and Rodríguez-Barreras and Sonnenholzner 2015 in *T. ventricosus*), possible differences in the response of different species of echinoids against intra-coelomic tagging devices should be a reasonable explanation. It is possible that *Diadema antillarum* is more sensitive to invasive devices, which could still be used in other more tolerant species, such as *T. ventricosus* (Rodríguez-Barreras and

Sonnenholzner 2015). Further experiments are needed, involving other tropical and temperate sea urchin species to reassess these results.

The development of a suitable tagging method is a real need for long-term studies in *D. antillarum*. Differences between previous studies conducted under controlled conditions and field experiments reflect the high variability and the necessity of testing tagging procedures under both conditions. The present study does not support the use of any of these tags for long-term studies in *D. antillarum* due to their low retention. The S-tag and T-bar were the least adequate tags to use in *D. antillarum*. The S-tag induced high spine autotomy; whereas the T-bar tags had low retention, and survival. Nevertheless, T-bars can be useful and cheap for short-term studies on *Diadema* (see Clemente *et al.* 2007). We do not recommend PIT-tags for short-term studies because of their relatively low effectiveness with *D. antillarum*. Although retention rates of PIT-tags were significantly higher than the other two tags, it is nevertheless, too low to make them useful for long-term field experiments.

Arrested recovery of *Diadema antillarum*
population: survival or recruitment
limitation?



ABSTRACT

Densities of the long-spined urchin *Diadema antillarum* remain significantly below pre mass mortality levels at most Caribbean localities. The arrested recovery of this formerly abundant and important herbivore has been attributed to low supply of recruits, or high post settlement mortality. There is, however, debate as to which of these factors is determinant of the local dynamics of this echinoid. In this study we use demographic modeling to analyze the contribution of recruitment and post settlement survival on the dynamics of *D. antillarum* in four localities in Puerto Rico. Our results indicate relatively high adult survival, and low stasis but high growth transition in the small individuals. The four populations exhibited asymptotic growth rates (λ) below 1.0, with lambdas varying from 0.918 to 0.964. Elasticity analysis indicates that the survival of large sized echinoids can potentially contribute most to changes in λ for all sites. Recruitment rate was low and exhibited high spatial and temporal variability. Numerical projections of the populations indicate that no site would exhibit an increase in density under current recruitment rates, but doubling recruitment would result produce growth in three of the four populations. We conclude that local recovery requires the spatial and temporal co-occurrence of high recruitment and survival rates.

INTRODUCTION

Caribbean reefs systems have deteriorated dramatically over the last decades (Gardner *et al.* 2003, Hughes *et al.* 2010, De'ath *et al.* 2012). Their decline has been associated with the cumulative effect of natural and human-induced factors such as, hurricanes, disease outbreaks, bleaching, pollution, and overfishing (Hughes 1994, Bythell *et al.* 2000, Kramer *et al.* 2003). One of the most significant shifts in benthic community structure of Caribbean reefs occurred after the mass mortality event of the long-spined sea urchin *Diadema antillarum* (Philippi 1845) caused by an unknown pathogen between 1983 and 1984, and which caused mortality rates of up to 99.9 % at some Caribbean localities (Hughes *et al.* 1985, Hunte *et al.* 1986, Liddel and Ohlhorst 1986, Lessios *et al.* 1988, Lessios 2013).

During the thirty years since this region-wide collapse, abundance of *D. antillarum* have remained well below pre-mortality levels (Lessios 1988, Karlson and Levitan 1990, Levitan *et al.* 2014). Most studies report densities that still remain around or below 1 urchin m⁻² (Carpenter and Edmund 2006, Miller *et al.* 2007, Chiappone *et al.* 2008, Ruiz-Ramos *et al.* 2011, Rodríguez-Barreras *et al.* 2014a). The lack of recovery of the formerly abundant *D. antillarum* (Levitan *et al.* 2014) has been attributed to low supply of recruits, or to high post settlement mortality (Lessios 1988, Tuya *et al.* 2007, Rogers and Lorenzen 2008, Harborne *et al.* 2009). Recruitment measurements, before and after the die-off event, indicated a decrease of settlement in the Caribbean after the mass mortality event (Bak 1985, Vermeij *et al.* 2010). This decrease has been attributed either to reduced fertilization success (Lessios 1988, however see Williams *et al.* 2010), or lack

of suitable conditions for larvae settlement (Rogers and Lorenzen 2008). Seaweeds produce tannins, phenols, and other secondary metabolites against herbivores that affect fertilization success, larvae survival, and settlement (Pennington 1985, Hay and Fenical 1988). After the collapse of *D. antillarum* populations, fleshy-algae have become overabundant in reef systems in the Caribbean (Carpenter 1985, Liddell and Ohlhorst 1986) but see Bruno *et al.* (2009), potentially compromising settlement.

Evidence in support of the role of post-settlement mortality in controlling the dynamics of *D. antillarum*, is the observation that lower abundances have been reported inside no-take reserves (Chiappone *et al.* 2002, Brown- Saracino *et al.* 2007, Harborne *et al.* 2009), whereas overfished areas report higher *Diadema* densities (Sellers *et al.* 2009, Idjadi *et al.* 2010). *Diadema* has multiple predators, including fish of the families Balistidae, Haemulidae, Labridae and Sparidae, lobster of the genus *Panulirus* spp., and gastropods of the genus *Cassis* spp. (Randall *et al.* 1964, Serafy 1979, Carpenter 1984). There is also evidence that physical factors can contribute to explain differences in *D. antillarum* abundance. The genus *Diadema* is susceptible to hydrodynamic forces because morphological features -long spine canopy- do not allow a large adhesive area to attach to the bottom (Tuya *et al.* 2007). Therefore, areas with low wave energy would tend to support higher abundance of *Diadema* (Debrot and Nagelkerken 2006, Clemente and Hernández 2008, Rodríguez-Barreras *et al.* 2014a).

Teasing apart the relative effect of recruit supply and post settlement mortality on the local dynamics and structure of open marine populations is not a trivial task. Caley *et al.* (1996) suggested multifactorial field experiments as a means of unraveling the effect of recruitment versus post-settlement mortality. Another approach towards the same end is

demographic modeling. Pfister (1996) and Hughes and Tanner (2000) successfully used this approach to analyze the effect of survival (among other vital rates) and recruitment on the population dynamics of a guild of tide pool fishes in the Pacific Northwest and three Caribbean corals, respectively. In this study we follow the demographic modeling approach to examine the apparent lack of population recovery of *D. antillarum*. We analyze the contribution of recruitment and post settlement survival to the dynamics of *D. antillarum* by constructing, parameterizing and analyzing size-based matrix population models for four localities of northeastern Puerto Rico Archipelago. We use the equations to numerically simulate the effect of different rates of recruitment on the local dynamics of the species. Thus, we evaluate the relative contribution of all matrix transitions (including survivorship) to population asymptotic growth rate (λ) through elasticity analysis.

METHODS

Site description and data collection- The field work was conducted at four shallow-water fringing reefs (< 3.0 m depth), located in northeastern Puerto Rico Archipelago: Cerro Gordo (CGD- 18°16'51.40"N, 65°17'12.21"W), Luquillo (LQY-18°23'18.46"N, 65°43'5.52"W), Tamarindo (TMD- 18°18'55.29"N, 65°19'5.82"W), and Melones (MLN- 18°18'15.70"N, 65°18'42.27"W) (Fig. 1). Geological composition of TMD and MLN is characterized by volcanic and intrusive rocks, with some limestone deposits, whereas LQY and CGD are made up by carbonate rocks. Structural complexity varies from sites with relatively high rugosity indexes (CGD and MLN), to less complex fringing reefs (TMD and LQY). Fleshy macroalgae had relative low cover with an average of less than 10 ± 6.37 % in the four localities, whereas live coral coverage was greater than 15% at all

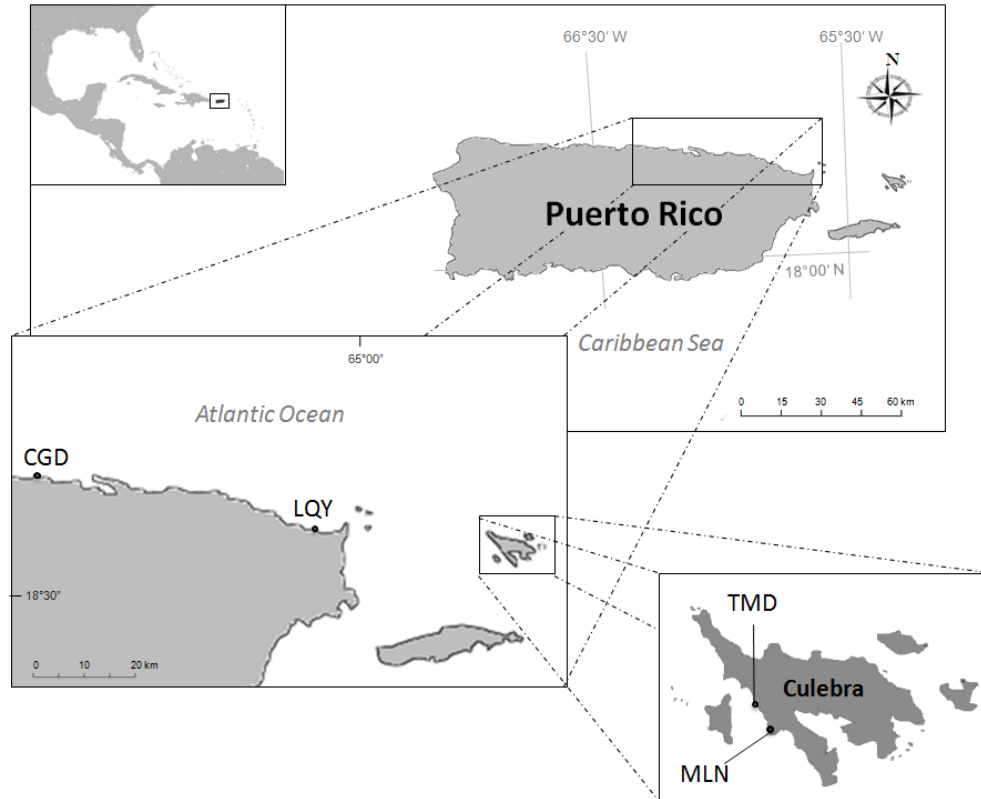


Figure 1 Study zone and sampling sites in Puerto Rico. Cerro Gordo (CGD), Luquillo (LQY), Tamarindo (TMD), and Melones (MLN).

sites, peaking at TMD with 29.09 ± 12.15 %. See more details in Rodríguez-Barreras *et al.* (2014a). Surveys were conducted during August of 2011, 2012 and 2013, and February of 2012 and 2013 (five censuses). Eight transects per site of 10 m^2 (5 m x 2 m) were established parallel to the coast and separated by 10 m each. Mean density was estimated using the belt-transect methodology (Sellers *et al.* 2009). Size frequency data was also collected during this period. Dataset details appear in Rodríguez-Barreras *et al.* (2014a), where all sites acronyms coincide, except TMD that is named TM1.

Demographic model- The life cycle of *D. antillarum* was summarized into three size classes: small, medium, and large individuals (Fig. 2). The small size class includes urchins with a horizontal test diameter between 10 - 40 mm; the medium size class

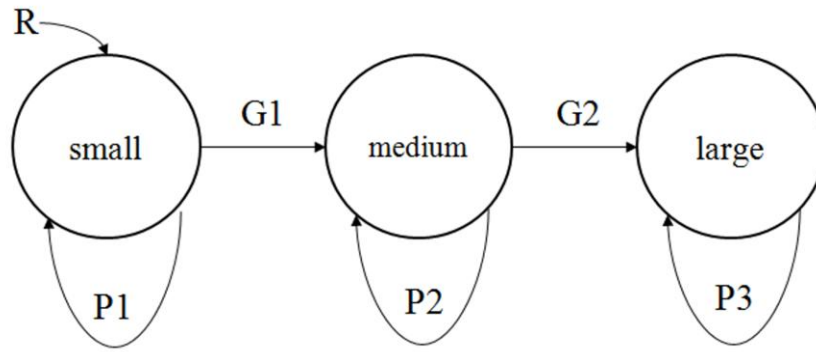


Figure 2 Size-based life-cycle graph of the sea urchin *Diadema antillarum*. Arrows indicate potential transitions between size classes. Transitions include the probability of surviving and remaining in the size class (P_x), the probability of surviving and growing from a given stage to the next one (G_x), and recruitment (R).

includes those larger than 40.1 and smaller than 60 mm, and the large ones are those with diameters in excess of 60.1 mm (Karlson and Levitan 1990, Miller *et al.* 2003, Rodríguez-Barreras *et al.* 2014a). Data on larval stage of *D. antillarum* is scarce, and the few available studies have focused in measuring abundance in the pelagic stage, but not of survival or settlement success in natural reef substrate (See Vermeij *et al.* 2010, Williams *et al.* 2010). Early recruits were also excluded because the cryptic behavior and small size of this early stage does not allow an accurate estimate of their abundance. Thus, the simplified life cycle we were analyzing in this study excludes the pelagic phase, and the initial 2-3 months after settlement (assuming a growth rate of between 6-7 mm.month⁻¹ (Levitan *et al.* 2014). We assumed in this analysis that the number of individuals entering the population at a test size of 10 mm is more strongly influenced by the number of larvae successfully settling at each site, than the number removed by predators during the first 4 to 6 weeks after settlement is relative low because of their cryptic behavior, and refugee availability (Levitan *et al.* 2014).

The demographic model in equation 1 presents five transitions: the probability of surviving and remaining in the same size class (P_x), and the probability of surviving and growing (G_x) from a given stage to the next one.

$$\begin{bmatrix} S \\ M \\ L \end{bmatrix}_{t+1} = \begin{bmatrix} P1 & 0 & 0 \\ G1 & P2 & 0 \\ 0 & G2 & P3 \end{bmatrix} \times \begin{bmatrix} S \\ M \\ L \end{bmatrix}_t + \begin{bmatrix} R \\ 0 \\ 0 \end{bmatrix}_t \quad \text{Eq. 1.}$$

These transitions were estimated using the quadratic programming routine (QP); a mathematical algorithm that minimizes quadratic forms subjects to inequalities, using changes in the abundance of the three size classes, during the three censuses. This procedure takes as inputs the C matrix (constrain -non-negative), and the vector $b = (0 \ 0 \ 0 \ 0 \ 0 \ 1 \ 1 \ 1)$. A new matrix G and a new vector f are generated and used in the QP routine that returns a p vector with the demographic parameters ($P1$, $G1$, $P2$, $G2$, and $P3$). See Caswell (2001) and Stubben and Milligan (2007) for other QP details. This routine was initially developed for close-populations, and one limitation is that mortality (μ) for the whole life cycle is only reflected in the last size class. That is, small and medium urchins either stay in their size class with probability P_x or advance to the next one with probability G_x , none die (i.e. $P1+G1=1$ and $P2+G2=1$). In order to incorporate mortality in the small and medium size classes, the estimated μ was divided equally among the three size classes (see Appendix Tab. A1). This decision is based in the premise that *D. antillarum* populations have a stable size distribution and size-independent mortality (Levitan *et al.* 2014).

Another simplification of this model is that it does not consider size retrogressions (i.e. the probability of an individual reducing its body size and returning to a previous size-

class). Shrinkage has been shown to occur in *D. antillarum*, but under experimental densities of 12, 24, and 48 urchin m⁻² (Levitan 1988). These densities, though, are far from current densities of *Diadema* in the Caribbean and Puerto Rico since 1984 (Lessios 2013). In fact, none of our sites exceeded an average density of 3 ind.m⁻² (Rodríguez-Barreras *et al.* 2014a), which is three times lower than the lowest density used by Levitan (1988). Retrogressions are associated to food limitation (Sebens 1982), and given the current low densities it is reasonable to assume that *D. antillarum* populations are not resource limited.

Recruitment was estimated by dividing the number of small sea urchins for each census period by the sampling area of 80 m². Recruitment (R) was included outside the projection matrix as a column vector, to reflect the open nature of *Diadema* populations. This sea urchin has a bipartite life history in which benthic adults produce pelagic larvae that can live as part of the zooplankton, between 30 up to 60 days (Leber *et al.* 2008). This allows larvae disperse distances of hundreds kilometers (Hunte and Younglao 1988).

The real dominant eigenvalue and the corresponding right and left eigenvectors of the 3 x 3 projection matrix were calculated to obtain the asymptotic population growth rate (λ), plus the stable stage and reproductive value vectors. The stable stage and reproductive value vectors were used to calculate the elasticity matrices (Caswell 1996). Asymmetric 95% confidence limits of λ were estimated from the 2.5th and 97.5th percentiles with a bootstrapping routine 1000 times (Caswell 2001).

Simulations- Populations were projected by iterating equation 1 with a horizon of three decades under three different recruitment scenarios: observed, half, and double, and using

the observed size structure and abundance of each site as the initial population vector. Results are presented in terms of density (urchin m⁻²). Chi-square analyses were performed to compare observed versus calculated size frequency distributions (Zar 2010). Statistical analyses were performed in the free-license statistical program R-3.0.1 with a p value of 0.05, <http://www.r-project.org/> (R Core Team 2013). Demographics analyses were run on R using the package popbio (Stubben and Milligan 2007).

RESULTS

Population structure at all sites was dominated by the large-sized urchins, followed by medium and small ones, except in TMD where medium and large echinoids exhibited similar abundances (Fig 3). The life cycle of *D. antillarum* was characterized by relatively high values of stasis (i.e. surviving and remaining in the same size category) in the medium and large size classes. Low stasis and high transition rates were estimated for small sea urchins. Growth transitions between small and medium echinoids varied from 0.32 to 0.91, and 0.09 to 0.25 between medium and large ones (Tab. 1). On the other hand, recruitment was very variable in time. It was highest during 2013 with a pooled average for all sites of 0.12 ± 0.09 recruits/ year⁻¹/m⁻², and lowest during 2011 with an overall average of 0.083 ± 0.051 recruits/year/m⁻². High spatial variability was also observed, with the following order: CGD>TMD>MLN>LQY. The highest recruitment was observed at CGD (0.26 recruits/ year⁻¹/m⁻²), while the lowest rate was observed at LQY with 0.02 recruits/ year⁻¹/m⁻² (Fig. 4).

Average mortality was $16.1\% \pm 0.06$, ranging from a minimum of 13.6 reported in MLN, and a maximum of 24.7% reported in CGD (Tab. 1). The four populations exhibited

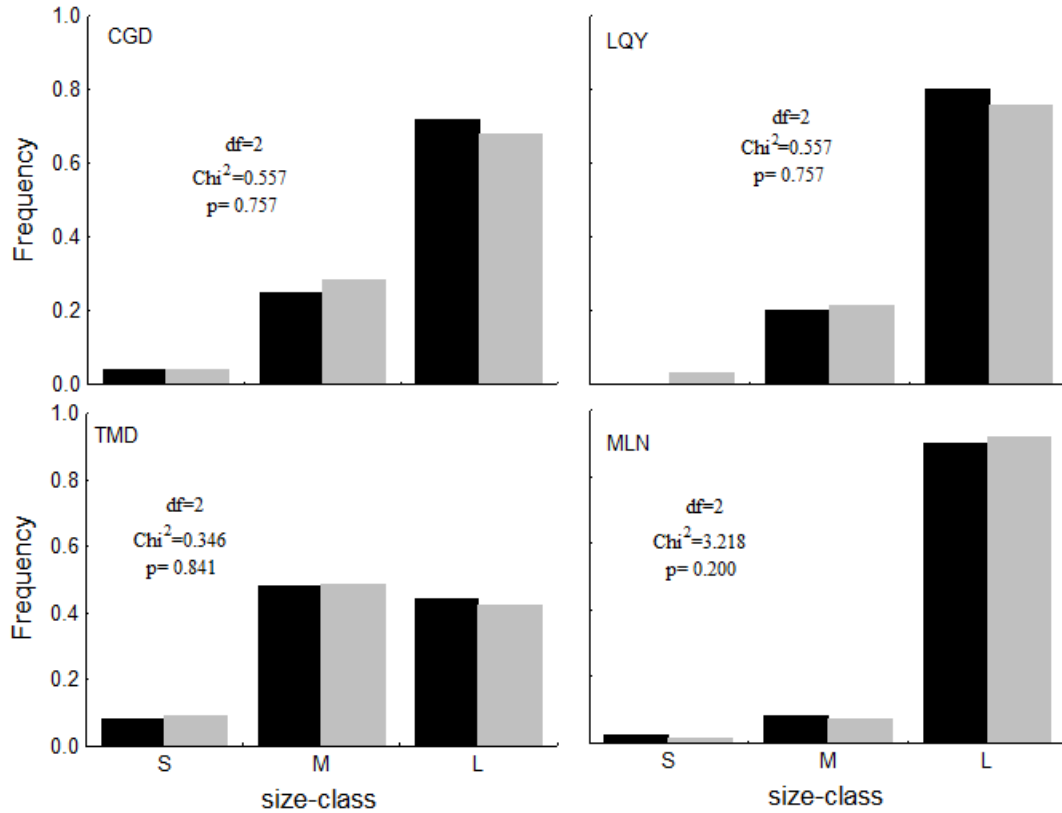


Figure 3 Observed (black bars) and expected (gray bars) size structure of *Diadema antillarum* at the four study sites. Expected structure is the stable stage vector calculated from matrices in Table 1. The size class categories were small (10-40 mm), medium (40.1 - 60 mm), and large (>60.1 mm).

mean asymptotic growth rates (λ) below 1.0. These λ s, calculated from the matrices in Table 1, do not include the effect of recruitment (analyzed below), and should be interpreted as the rate of depletion of a cohort of recruits as they progress through the life cycle. Lambdas varied from a minimum of 0.918 at CGD to a maximum of 0.964 at LQY. There were no significant differences in the asymptotic growth rates among sites (i.e. no overlap in the 95% confidence intervals), except between CGD and MLN. As discussed above, this indicates unique survival and growth transition patterns among three of our sites. The stable stage structure calculated for each site (using the matrices in Table 1) was very similar to the observed size structure for each site (Fig. 3).

Table 1 Annual transition and elasticity matrices of the sea urchin *Diadema antillarum* in four sites of Puerto Rico. Elements > 0.1 are indicated in bold. Mean asymptotic population growth rates \pm 95% of confidence intervals estimated from bootstrapping.

Sites		Transition			Elasticity		
		S	M	L	S	M	L
CGD	S	0.012	0	0	0.001	0	0
	M	0.905	0.831	0	0.047	0.289	0
	L	0	0.087	0.918	0	0.030	0.586
	λ	0.918	c.i.	0.900	0.935		
LQY	S	0.500	0	0	0.041	0	0
	M	0.464	0.828	0	0.044	0.189	0
	L	0	0.136	0.964	0	0.031	0.657
	λ	0.964	c.i.	0.952	0.975		
TMD	S	0.617	0	0	0.052	0	0
	M	0.324	0.797	0	0.030	0.079	0
	L	0	0.144	0.941	0	0.025	0.784
	λ	0.941	c.i.	0.926	0.955		
MLN	S	0.441	0	0	0.033	0	0
	M	0.514	0.704	0	0.042	0.100	0
	L	0	0.250	0.955	0	0.036	0.746
	λ	0.955	c.i.	0.941	0.967		

Elasticity analyses performed to evaluate the proportional contribution of each matrix element to asymptotic population growth rate also exhibited site-specific patterns. The stasis of large sized echinoids can potentially contribute most to changes in λ at all sites (Tab.1). The contribution of survival and growth of small sized echinoids was insignificant at all sites, as well as the transition from medium to large ones.

In order to analyze the effect of recruitment on the local dynamics, equation 1 was iterated with a time horizon of three decades for each site. Under current recruitment

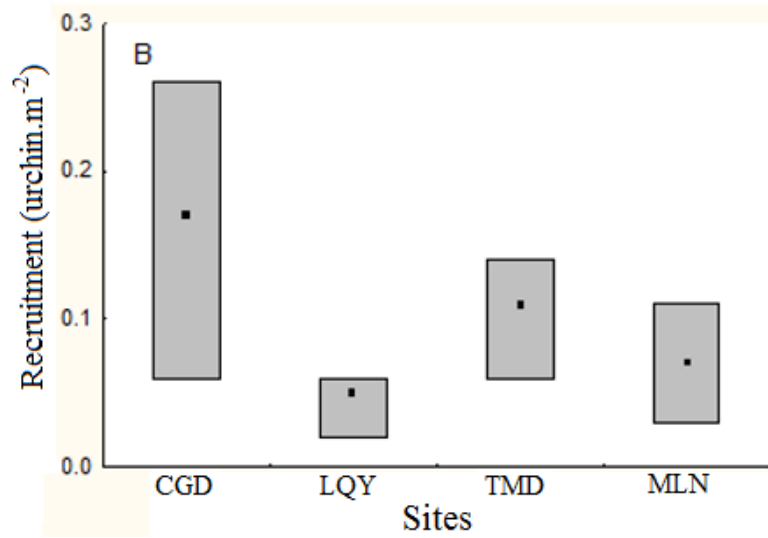


Figure 4 Average recruitment rates of the sea urchin *Diadema antillarum* of sites, with minimum and maximum form the four study sites (see map).

rates, all sites exhibited a decrease in density, with TMD stabilizing earlier than then other sites. Doubling recruitment favor growing at all sites, except in CGD, where *D. antillarum* abundance exhibited a small decrease and stabilized in 1.3 urchin m⁻² (Fig. 5). To further analyze the effect of recruitment on local dynamics we plotted the projected population density after 30 years as a function of recruitment for the four study sites. The slope of these lines can be understood as the sensitivity of local density to variability in recruitment. The figure 6 clearly demonstrates an interaction (i.e. heterogeneity of slopes) between recruitment and post settlement vital rates (represented by the λ of each site) on urchin local abundance. If the sites are ranked in terms of this measure of sensitivity (LQY > MLN > TMD > CGD), it corresponds exactly with the rank based on λ .

DISCUSSION

Large population fluctuations are more common in echinoderms than is usually thought. In fact, echinoderms have been called a “boom-bust” phylum due to several documented

crash and outbreak episodes (Uthicke *et al.* 2009). Nonetheless, three decades after the collapse (“bust”) of the sea urchin *D. antillarum*, the “boom” is yet to occur (Levitan *et al.* 2014). And even though long term abundance data is scarce (Lessios 2013), the arrested recovery in *D. antillarum* appears atypical. For instance, the holothurian *Amperima rosea* recovered from a population collapse in just one year (Billett *et al.* 2001), while the asteroid *Acanthaster planci* exhibited a delay of 15 years between two periods of outbreaks (Brodie *et al.* 2005). The only other documented example of arrested recovery in echinoderm is in the asteroid *Heliaster kubiniji*, a large predator which collapsed abruptly in 1978 in the Gulf of California due to an unknown cause (Dungan *et al.* 1982). There was no evidence of recovery four years later, and its current population status is unknown (Uthicke *et al.* 2009).

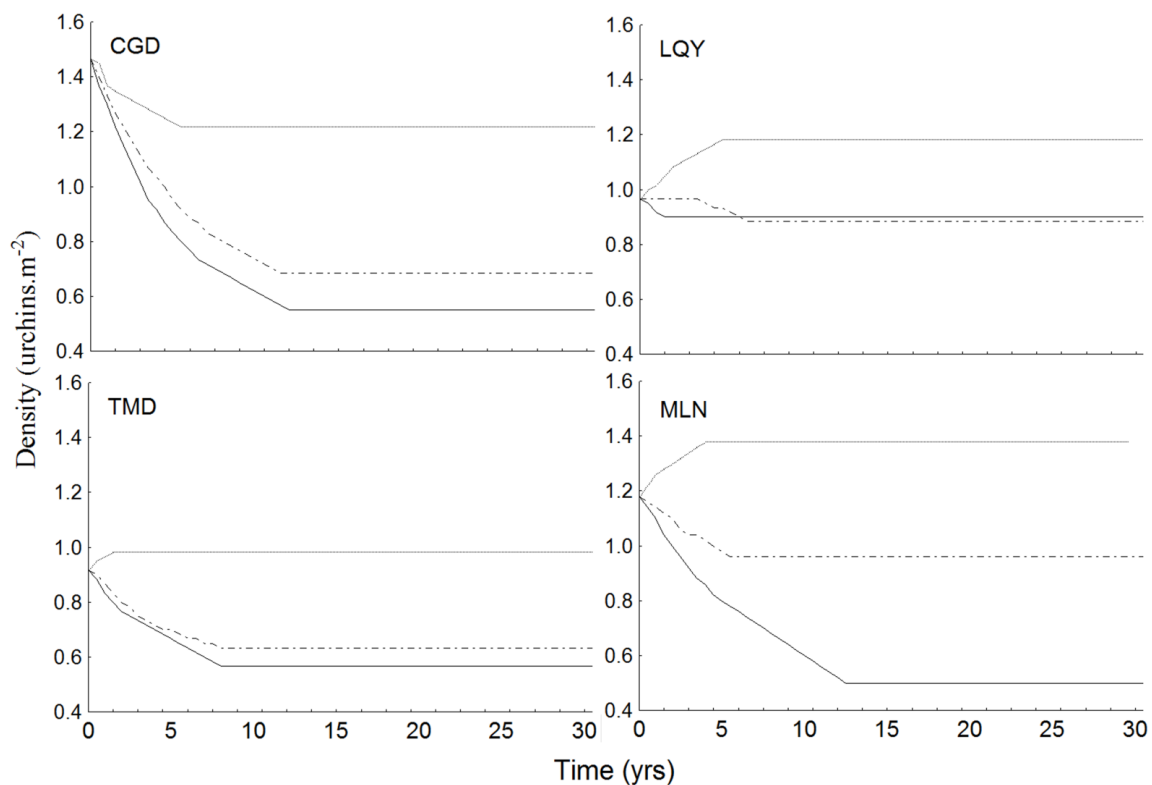


Figure 5 Projected densities of four populations of the sea urchin *Diadema antillarum* for different recruitment rates, observed average recruitment $x/2$ (bold line), x (dots and dash line), and $2x$ (light line), and. See method section for acronyms.

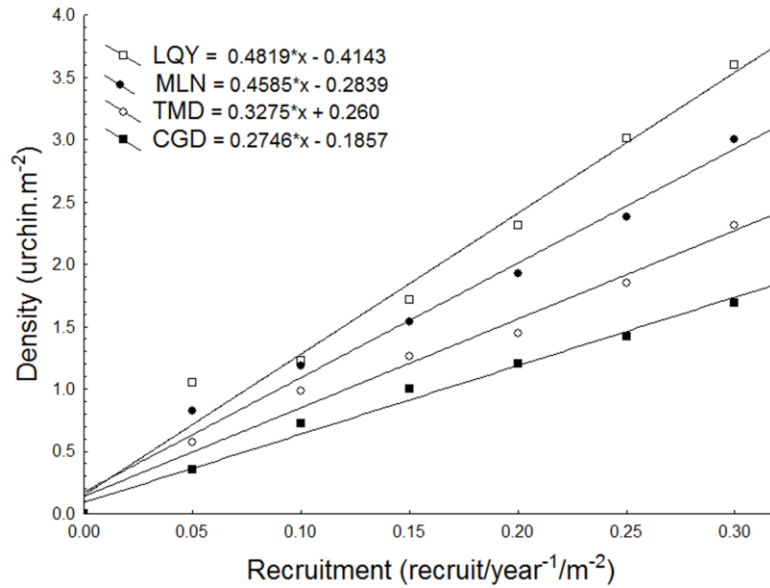


Figure 6 Simulated relationship between recruitment variability and urchin density at the four study sites (see methods for acronyms). Regression equation for each site is provided.

Based on the vital rates estimated in this study, *D. antillarum* can be characterized as a species with relatively high adult survival. This is in agreement with other studies conducted in *Diadema* where authors have found high survival rates (Karlson and Levitan 1990, Clemente *et al.* 2007, Levitan *et al.* 2014). In fact, high adult survival appears to be the norm in echinoderms (McPherson 1968, Ebert 1982, White *et al.* 1985, Ebert and Pussell 1992, Medeiros-Bergen and Ebert 1995, Lamare and Mladenov 2000, Morgan *et al.* 2000, Ebert 2007). Elasticity analysis of the projection matrices in Table 1 revealed that survivorship of large sized individuals, in general, can potentially contribute between 58.6 and 78.4%, depending on locality, to future changes in λ . This suggests the survival of adult echinoids as the most important factor determining the local species dynamics. This conclusion, however, should be interpreted with caution because the elasticity analysis does not consider the effect of recruitment.

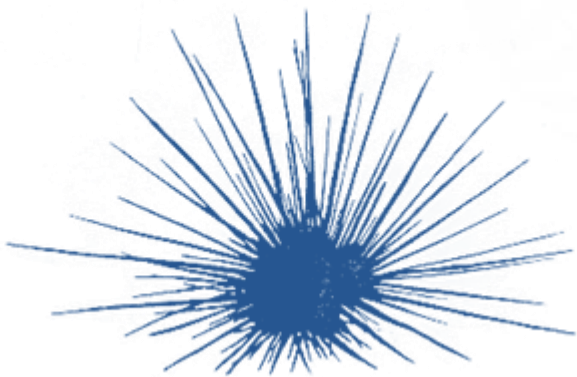
Reproduction was not included within the transition matrix in equation 1, but as a recruitment vector. This precludes elasticity analysis of reproduction based on the right and left eigenvectors of the transition matrix (Caswell 2001). Recruitment of *Diadema* in the study sites ranged between 0.02 and 0.26 recruits/year⁻¹/m⁻². This is somewhat lower than recruitment levels reported by Levitan *et al.* (2014) for U.S.V.I. (0.14 - 0.42 recruits/year⁻¹/m⁻²). These values reflect a severe recruitment deficit at regional scale. Other studies have measured recruitment of *Diadema* sp.; however, the methodology used in these studies does not allow reasonable comparisons with our results (Lessios 1995, Edmund and Carpenter 2001, Tuya *et al.* 2006, Hernández *et al.* 2010, Williams *et al.* 2010).

The numerical simulations using equation 1 show that even with the observed relative high survival of adults, current recruitment levels are not sufficient for *D. antillarum* populations to surpass a density of 2 urchin m⁻². Doubling recruitment rates would lead to an increase in density in three of our four sites. Only CGD exhibited a projected decline in current density under the 2x recruitment scenario, which seems to be related with its relatively low adult survival rate (Tab. 1). This has important implications from a management perspective because it means that sites with high adult mortality would benefit more from an increase in post settlement survival (i.e. moving vertically in Figure 6) than from an increase in recruitment (i.e. moving horizontally); whereas sites with high adult survival would be the only ones benefiting from recruitment pulses.

This interaction between recruitment and post settlement vital rates is not unique for *D. antillarum*; it has also been documented and discussed by authors working with other populations of marine species with sedentary adults and pelagic larvae (e.g. Pfister 1996,

Hughes and Tanner 2000). In fact, the controversy regarding the causes for the lack of recovery of *D. antillarum* can be better understood if viewed in terms of this interaction. Our model predicts that sites with high adult survivorship ($> 95\%$) and recruitment rates $> 0.15 \text{ year}^{-1}/\text{m}^{-2}$ are expected to reach the threshold of 2 urchin m^{-2} to avoid overabundance of fleshy algae in Caribbean reef systems (CARICOMP 2000 cited by Alcolado *et al.* 2013, Steiner and Williams 2006). The fact that very few localities in the Caribbean currently exhibit densities above 1 or 2 urchin m^{-2} indicate that spatial and temporal co-occurrence of high recruitment and high survival rates, are still rare after three decades of the mass-mortality event.

Effect of fish removal in the abundance and
size structure of the sea urchin *Diadema*
antillarum: a field experiment



ABSTRACT

Three decades after its population collapse, the sea urchin *Diadema antillarum* has yet to return to pre-mortality densities in most Caribbean localities. Circumstantial evidence suggests high post-settlement mortality due to predation as a potential mechanism to explain the lack of, or the slow recovery of this sea urchin. In this study we experimentally reduced the densities of two reef fish, *Thalassoma bifasciatum* and *Halichoeres bivittatus*, at one site for one year and measured the effect of their removal in the abundances and size structures of *D. antillarum* in relation to that of a comparable control site. We hypothesized a significant increase in urchin abundance and a reduction mean test diameter at the experimental site with respect to the control site after the reduction in fish abundance. Overall, the density of *D. antillarum* did not vary significantly between control and experimental sites for the different census periods. Fish removal had a significant effect on the size structure of *D. antillarum* and in the mean density of the medium size classes. Results suggest that removal of these two labrids can significantly increase the relative abundance of medium-sized sea urchins and modify its population structure. Efforts to recover *D. antillarum* populations would be strengthened if densities of *T. bifasciatum* and *H. bivittatus* remain low.

INTRODUCTION

The grazing activity of sea urchins and herbivorous fish is essential for the functioning of coral reef ecosystem through structuring of algal assemblages and nutrient recycling (Carpenter 1986, Clemente et al. 2010). Both groups of herbivores have declined significantly in the Caribbean as a consequence of either overfishing of parrotfishes (Jackson *et al.* 2012) or epizootic events in the case of urchins (Lessios 2013). In particular, populations of the Caribbean long-spined sea urchin *Diadema antillarum* Philippi, 1845 which was once a very abundant species with densities up to 23 individuals per m² or higher (Scoffin *et al.* 1980), suffered a region-wide collapse in the early 1980's as a consequence of an unknown pathogen (Lessios 2013). Three decades later, populations of *D. antillarum* have not returned to the pre mass mortality densities in most Caribbean localities (Levitan *et al.* 2014; Rodríguez-Barreras *et al.* 2014). Deficiency of recruits (Miller *et al.* 2009), unsuitable conditions for larval settlement (Roger and Lorenzen 2008), limited dispersal due to a combination of low densities of adults and poor water quality (Ruiz-Ramos *et al.* 2011), and high post-settlement mortality caused by predation (Lee 2006; Harborne et al. 2009) are among the mechanisms proposed to explain the arrested recovery of *Diadema*.

The direct and indirect effects of predators on lower trophic levels can regulate the relative abundance of species within communities (Paine 1966, Estes *et al.* 1998, Freeman 2006). The effects of predation upon sea urchin populations, habitat selection, reproduction, movement pattern and feeding behavior have been extensively studied in both tropical and temperate ecosystems (Lewis 1964, Carpenter 1984, Sala *et al.* 1998,

Tuya *et al.* 2004, Martín and Luque 2008, Kintzing and Butler 2014). For example, McClanahan (2000) documented a 50% reduction of biomass of the sea urchin *Echinometra mathaei* Blainville, 1825) in Kenyan reefs in less than ten years after recovery of the predator *Balistapus undulates* (Park, 1797). Similarly, Estes and Palmisano (1974) showed that areas with more sea otters (20-30 ind/km²) had fewer and smaller sea urchins of their preferred prey than areas with fewer otters.

Sea urchins are prey for multiple predator species that vary across habitats and ecosystems. In the Caribbean, predators of *D. antillarum* include fishes of the families Balistidae, Haemulidae, Labridae, Sparidae, lobsters and some molluscs (Randall *et al.* 1964, Serafy 1979, Carpenter 1984, Levitan and Genovese 1989). Labrids, in particular, are primarily benthophagous feeders, well known for their high foraging intensity (Clifton and Motta 1998). *Halichoeres bivittatus* (Bloch, 1791) and *Thalassoma bifasciatum* (Bloch, 1791), the predator species in this study, are considered among the most abundant fish on Caribbean coral reefs (Nelson 2006). They are active only during daytime and eat a wide range of food resources, such as plankton, eggs, and fish ectoparasites (Feddern 1965). Both species have been listed as potential predators of *D. antillarum* juveniles (Lessios 1988), and fragments of spines and test of *Diadema* have been collected in stomach content analyses (Randall *et al.* 1964).

Observational and experimental data indicates that predation on juvenile and early adult stages may influence population size structure, distribution, and abundance of sea urchins (Scheibling and Hamm 1991, Sala and Sabala 1996, Hereu *et al.* 2005, Clemente *et al.* 2007a). For example, small individuals of *Diadema* displayed size-dependent mortality before reaching 40 mm of horizontal test diameter (Clemente *et al.* 2007a). Furthermore,

low and variable population densities of *D. antillarum* inside and outside fishing reserve areas of the Florida Keys were believe to be a function of predation rate and competition with herbivorous fish (Chiappone *et al.* 2000). In addition, higher densities of *D. antillarum* have been recorded in overfished areas where sea urchin predators have been significantly reduced (Edmunds and Carpenter 2001, Idjadi *et al.* 2010). These and other studies (Brown-Saracino *et al.* 2007, Harborne *et al.* 2009, Martín-Blanco *et al.* 2010) provide evidence in support of predation regulating the abundance of *D. antillarum* populations. Nonetheless, the effect of predators on the abundance and size structure of *D. antillarum* remains to be demonstrated experimentally (but see Kintzing and Butler 2014).

In this study we experimentally reduced the density of *T. bifasciatum* and *H. bivittatus* for one year at one site and evaluated if these two labrids had an effect on the abundance and size structure of *D. antillarum* by comparing the results with a similar control site where fish density was not manipulated. If these two labrids are exerting a significant effect on *D. antillarum* through predation, we expect an increase in urchin abundance and a reduction in mean test diameter at the experimental site compared with the control after the reduction in fish abundance.

METHODS

Study sites- The experiment was conducted between Sept. 2013 and Sept. 2014, on two shallow-water reefs (≤ 2.0 m depth) located off the northeastern coast of Puerto Rico: Cerro Gordo in Vega Baja (18°16'51.40"N, 65°17'12.21"W), and Costa Azul in Luquillo (18°23'18.46"N, 65°43'5.52"W). Both localities are similar in depth, substrate type and

complexity, wave-exposure, and substrate coverage. See Rodríguez-Barreras *et al.* (2014) for more details on site characterization. We assigned Cerro Gordo as the control site, and Costa Azul as the treatment site. The study sites are very shallow (< 2m) back reef zones in areas characterized by heavy fishing pressure. Large sea urchin predators such as Sparids and *Balistes* were absent at both sites. Invertebrate predators of *Diadema antillarum* such as spiny lobster *Panulirus argus* (Latreille, 1804), or helmet snails (*Cassis* spp.) were also not recorded during the experiment.

Experimental design- We established one control (Cerro Gordo) and one treatment (Costa Azul), without replicates. The abundance of *Halichoeres bivittatus* and *Thalassoma bifasciatum* was reduced at the treatment site by capturing fish with a Folding Net with a telescopic handle of 150.0 cm in length, a net diameter of 40.5 cm and a mesh size of 0.4 cm. No fish removal was performed in the control site. Removal visits were conducted every two weeks from September of 2013 to September of 2014. All collected individuals were identified, measured, placed in suitable containers with an air supply, and released approximately 3 km from the treatment locality. The abundance of both fish species was estimated every three months within three stationary circular plots (5 m radius) over 5 minutes (Bortone et al. 1989). The number of individuals within the area was used to estimate the density of both fish species. Individuals of *Diadema antillarum* were divided into three size-class categories - small, medium, and large individuals. The small size-class included urchins with a test diameter smaller than 40.0 mm; the medium size-class included those larger than 40.1 and smaller than 60.0 mm, and the large one were those with diameters in excess of 60.1 mm (see Karlson and Levitan 1990, Miller *et al.* 2003, Rodríguez-Barreras *et al.* 2014).

Mean urchin densities were estimated using a belt-transect method (Sellers *et al.* 2009). At each site, we randomly established six transects of 10 m² (5 m x 2 m) parallel to the coast and separated by 10 m from each other. All individuals within transects were counted. Crevices and small holes were carefully inspected to avoid missing any small sea urchins. Additionally, we measured the test diameter of 50 individuals - selected randomly from the belt transects - with the use of a caliper (caliper error ± 0.05 mm).

Statistical analyses- Two-way ANOVA was used to test for differences in mean test diameter and mean densities of *Diadema antillarum* with ‘control vs. treatment’ and ‘time’ (i.e. census periods) as the independent variables. The model treats site as a fixed factor and time as a random factor. A power transformation was used to meet normality and homogeneity of variance assumptions (Box and Cox 1964). Size frequency data (sea urchin) was square-root transformed, whereas no transformation was required for mean densities of both fish wrasses and *D. antillarum*. A posteriori Tukey-HSD analyses were done for multiple comparisons of means among time periods for density and size (Zar 2010). A Chi² analysis was done to test for differences between initial and final urchin size structure in the treatment. The results of this experiment were analyzed using a two-way ANOVA with time (different census periods) and site/treatment as the independent variables. Due to difficulties in establishing the fish removal treatment and control at the different sites it is impossible to separate the effect of treatment and site in this experiment. We understand, however, that the sites are comparable, and the observed differences in the dependent variables can be attributed more to treatment than to site effects. All statistical analyses were run in the free-license statistical program R-3.0.1 with a p value of 0.05 (R Core Team 2014).

RESULTS

Fish density in the control site ranged between 0.34 ± 0.10 and 0.52 ± 0.14 ind.m⁻² for *Thallasoma bifasciatum*, and between 0.29 ± 0.02 and 0.63 ± 0.06 ind m⁻² for *Halichoeres bivittatus*. At the manipulated site, we recorded a significant reduction in the average abundance of both wrasse species after the first three months of the removal (Fig. 1). Average density of *H. bivittatus* was reduced by almost a third from 0.74 ± 0.10 to 0.26 ± 0.02 ind m⁻²; while the reduction for *T. bifasciatum* was four fold from 0.72 ± 0.06 to 0.15 ± 0.04 ind m⁻². We found significant differences in the abundance of both wrasses between sites and among census periods, with temporal differences being site-specific (Table 1). A posteriori analysis indicated that these differences between control and treatment were significant from December 2013 to the end of the experiment (Tukey-HSD test, $p < 0.01$). Thus, differences in mean densities of both wrasses remained significant among initial and subsequent censuses for both species at the manipulated site only ($p < 0.05$).

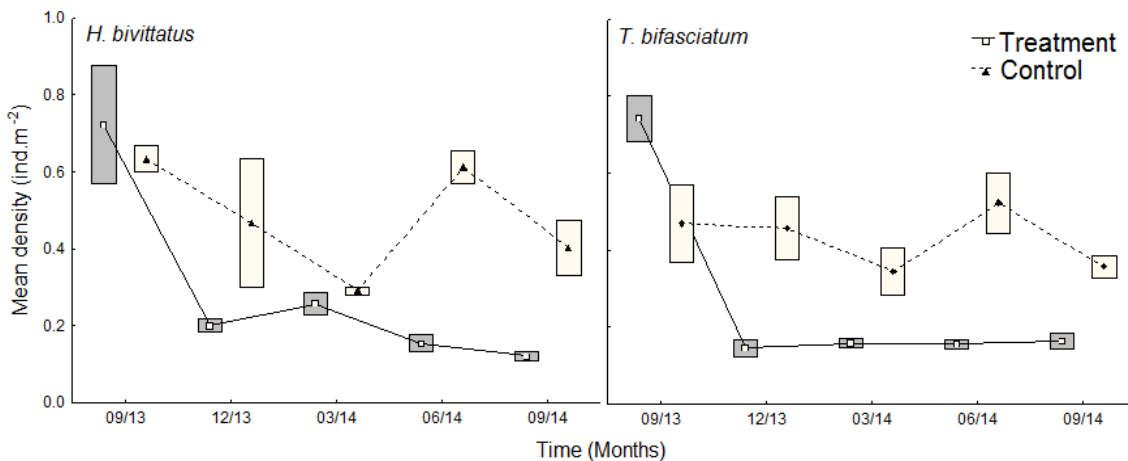


Figure 1 Abundances of (A) *Halichoeres bivittatus* and (B) *Thalassoma bifasciatum* for control and treatment (mean \pm standard error).

Overall mean density of *Diadema antillarum* was 1.23 ± 0.57 ind m^{-2} at the control site, ranging from 0.92 ± 0.55 to 1.47 ± 0.50 ind m^{-2} . At the experimental site mean density was 1.26 ± 0.18 ind m^{-2} , ranging from 0.97 ± 0.56 to 1.53 ± 0.36 ind m^{-2} . No apparent temporal pattern or trend was evident at the control site. However, a sustained increment of abundance from March to the end of the study was observed in the manipulated site (Fig. 2a). This trend, however, was not significant (Table 2). In fact, no significant differences in overall mean sea urchin abundance were found with respect to site/treatment, time, or their interaction (Table 2).

Table 1 Effect of time in the abundance of two wrasses *Halichoeres bivittatus* and *Thalassoma bifasciatum* in the control and the treatment. Asterisks indicate significance.

Control	<i>T. bifasciatum</i>					<i>H. bivittatus</i>				
	DF	SS	MS	F	p	DF	SS	MS	F	p
Time	4	0.07	0.02	1.08	0.42	4	0.25	0.06	2.89	0.08
Error	10	0.16	0.02			10	0.22	0.02		
Total	14	0.23				14	0.47			
Treatment										
Time	4	0.83	0.21	72.73	0.0001	4	0.73	0.18	11.93	0.001
Error	10	0.03	0.01		*	10	0.15	0.02		*
Total	14	0.86				14	0.88			

Variability in density of each size class was analyzed between treatments/sites, census periods (time) and their interaction. Site (df= 1, F=17.61, $p < 0.001$) and temporal (df= 4, F=16.58, $p < 0.001$) differences in the abundance of the small urchins were significant, as well as their interaction (df=4, F=9.81, $p < 0.001$). The abundance of medium-sized sea urchins showed a sustained increase in the treatment site while in the control site it fluctuated without any discernible pattern (Fig. 3). Site (df=1, F=7.89, $p = 0.07$) and temporal (df= 4, F= 2.07, $p = 0.038$) differences were detected for this size-class, and the

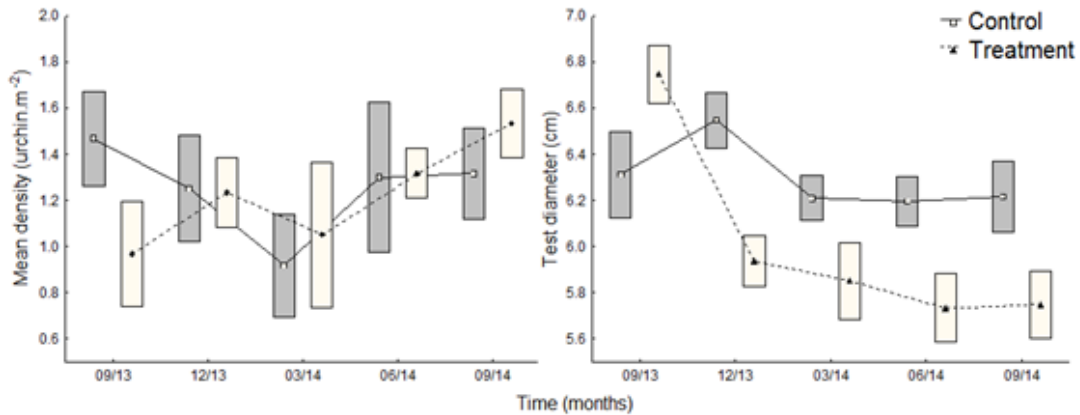


Figure 2 Average (A) abundances and (B) test diameters of *Diadema antillarum* for control and treatment (mean \pm standard error).

interaction between both factors was also significant ($df=4$, $F=8.43$, $p<0.001$). The abundance of the large-sized individuals exhibited spatial ($df=1$, $F=4.76$, $p=0.034$), but not temporal ($df=4$, $F=0.98$, $p=0.426$) differences. The interaction between time and site was not significant ($df=4$, $F=0.47$, $p=0.758$).

DISCUSSION

Predator-prey interactions between fishes and sea urchins have been documented world-wide for several coral reef ecosystems, and fishes have been shown to have a strong predatory effect upon different life cycle stages of echinoids (McClanahan and Shafir 1990, Clemente *et al.* 2010, Harborne *et al.* 2009). Large-sized coral reef predators of sea urchin include triggerfish (Fam. Balistidae), grunts (Fam. Haemulidae) and porgies (Fam. Sparidae), (Serafy 1979, Carpenter 1984, Levitan and Genovese 1989); whereas small-sized predators comprise mostly species of the Labrid family (Randall *et al.* 1964). The wrasses (labrids) are considered generalist carnivores feeding on a variety of small

invertebrates (Feddern 1965). The wrasses *Thallasoma bifasciatum* and *Halichoeres bivittatus* have been identified as potential predators on juveniles of *Diadema antillarum* (Lessios 1988). Labrids have a very variable diet (copepods, shrimps, isopods and echinoids, etc.) apparently related to species-specific body sizes, jaw morphology and food item availability (Randal 1967; Clifton and Motta 1998). Sea urchins have been reported to comprise between 1.5% and 17.2% of the diet of *T. bifasciatum* and *H.*

Table 2 Effect of time in the abundance and average size of the sea urchin *Diadema antillarum* for the control and treatment. Asterisks indicate significance.

bivittatus, respectively (Randal 1967).

Control	DF	Mean density				P	DF	Mean size				p
		SS	MS	F				SS	MS	F		
Time	4	0.99	0.25	0.71	0.59		4	6.68	1.67	2.11		0.08
Error	25	8.71	0.35				245	194.09	0.79			
Total	29	9.70					249	200.77				
Treatment												
Time	4	1.21	0.30	1.21	0.33		4	24.82	6.21	6.53		0.0001
Error	25	6.22	0.25				245	232.68	0.95			*
Total	29	7.43					249	257.50				

The highest current densities of *D. antillarum* have been reported in overfished areas such as Jamaica, and explained in terms of low abundance of predators (Sellers *et al.* 2009, Clemente *et al.* 2010, Idjadi *et al.* 2010). Conversely, densities of *D. antillarum* have exhibited reductions in no-take reserves where fish stocks have recovered (Brown-Saracino *et al.* 2007; Martin-Blanco *et al.* 2010; Mercado-Molina *et al.* 2014).

Nevertheless, small labrids (wrasses) are usually excluded and dismissed as potential predators of *D. antillarum* (see Harborne *et al.* 2009). Small fishes are controlled by larger piscivorous fishes (Carr and Hixon 1995), and under overfishing conditions

wrasses can become more abundant. This study provides the first experimental results demonstrating an effect of labrids (Labridae) upon the sea urchin *D. antillarum*.

Although no significant effect of the fish removal treatments were found with respect to overall urchin density, the experiment indicates a significant effect of *T. bifasciatum* and *H. bivittatus* on the abundance of medium-sized *Diadema*. In fact, after reducing the density of both labrids, we observed an increment in the density of medium-sized sea urchins that led to an overall reduction of test diameter at the experimental site (see Fig 2). The sea urchin *D. antillarum* is a very conspicuous species due to its extremely long and fragile spine canopy. This morphological feature provides an effective defense in large urchins against predators; nevertheless, it also constitutes a disadvantage because it reduces the availability of refuges and exposes medium-sized individuals to predators. An explanation for the lack of a significant response in spatial differences of small sea urchins may be related with the fact that small individuals have developed a cryptic behavior (Clemente *et al.* 2007b), and the small individuals have more refuge opportunities than medium ones, being less susceptible to the attack of wrasses.

The observed differences between sites in the abundance of small-sized sea urchins may be more related to limited availability of recruits than the effect of wrasses per se. Local reproductive output is unlikely to control local population growth because the input of new recruits is decoupled from local reproductive effort in open populations (Shima 2001). In fact, larvae of *D. antillarum* live as part of the zooplankton for between 30 and 60 days, and may disperse from source populations to settlement areas in the order of kilometers away (Leber *et al.* 2008). Thus, larval availability is not constant throughout the year (Williams *et al.* 2009) and is related to seasonal peaks in the reproductive cycle

of *D. antillarum* (Hunte and Younglao 1988). Although an increase in the abundance of small sea urchins was observed in both treatments (see Fig. 3), it remains unclear whether this increase is related to fish removal. Therefore, the small size class abundance could also be a result of temporal changes in the recruitment dynamics, more than the effect of predator removal per se.

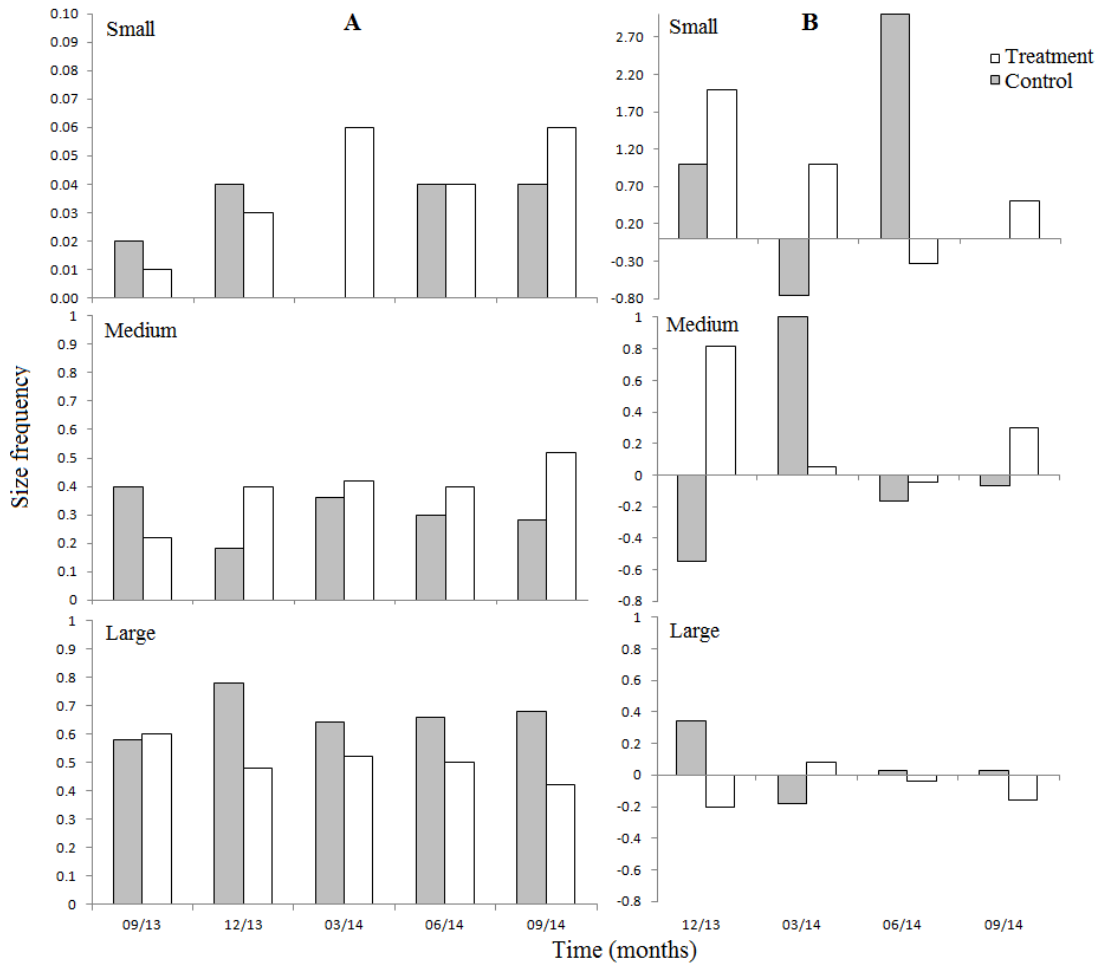


Figure 3 (A) Size structure of sea urchin *Diadema antillarum* for control (gray bars) and treatment (white bars) for the five census periods. (B) Change in abundance of small, medium and large *Diadema antillarum* for control (gray bars) and treatment (white bars) with respect to the previous census period. Notice that panels of the small category have different scales respect to the other categories. Frequency in Panel B was calculated as the number of individual t_{+1} / number of individual t .

Multiple stable states can develop in predator-prey models (see Ricklefs 2001). Under high density conditions, prey populations are more likely to be controlled by resource limitation, but with low densities, predators are able to exert their control on prey abundance more effectively (Ricklefs 2001). Before the collapse of *D. antillarum* in the 1980's, the population dynamic was probably controlled by local resource abundance (i.e. algal cover). However, after the demographic collapse, predators may have become more important in keeping *Diadema* populations in a low-density steady state. Although definite conclusions cannot be drawn from 1 yr of data, changes in population size structure were not seen before in the treatment site (see Rodríguez-Barreras *et al.* 2014). These may indicate that one year should be enough time to detect differences in size-class structure, and changes in the abundance of small and medium sized sea urchins. However, overall density, which is determined by the most abundant size class (i.e. the large urchins), should take more time to manifest.

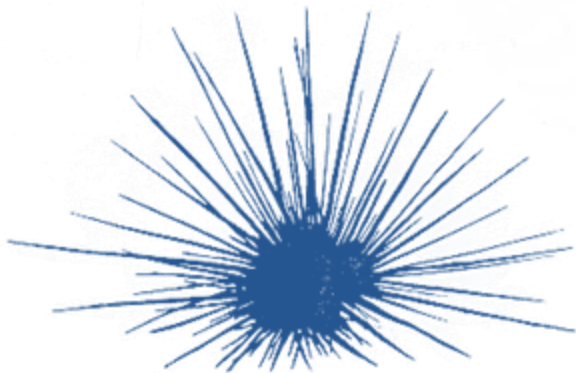
The wrasses *T. bifasciatum* and *H. bivittatus* are considered notorious opportunists. Randall *et al.* (1964) suggested that both species probably obtained their echinoid food by stealing from other large predators. Our results, however, indicate that the action of these wrasses appears to be more than a simple opportunistic response. Juveniles of *T. bifasciatum* are documented cleaner fish (Randall 1967). However, overfishing may have reduced the availability of its food resources, and increased pressure over remaining resources, such as *Diadema* and other small invertebrates. Thus, current densities of wrasses may be a result of long-term overfishing, that may reduce piscivorous fishes, and favor the overabundance of *Thalassoma* and *Halichoeres* spp. Further studies would be necessary to elucidate the effect of each size class of these wrasses on *D. antillarum* and

the effect of different fish community assemblages in the interaction between both labrids and *D. antillarum*.

After three decades of the mass mortality event, *D. antillarum* has yet to recover its pre mass mortality densities. This experiment suggests that removal of these two labrids can significantly enhance the relative abundance of medium-sized sea urchins and modify the species population structure. Therefore, any effort to recover *D. antillarum* populations would be strengthened if densities of *T. bifasciatum* and *H. bivittatus* remain low.

The background of the slide features a series of dynamic, flowing lines in various shades of blue and white. These lines originate from the left side and sweep across the frame towards the right, creating a sense of movement and depth. The lines vary in thickness and opacity, with some appearing as sharp, bright white streaks and others as softer, more blended blue washes.

General Conclusions & Recommendations



Three decades after the collapse of the sea urchin *D. antillarum* populations (Lessios 2013), our results indicate that current abundances of this urchin have not returned to pre-mortality levels. The lack of temporal differences between 2011 and 2013 are suggestive of a stable state of low density in Puerto Rico. However, population densities of the species showed some degree of recovery when we compare with previous studies conducted in the last decades (Weil *et al.* 2005, Ruiz-Ramos *et al.* 2011). The existence of spatial differences in densities among sites was related with the exposure to wave action. In fact, this factor seems to be determinant in the recovery process, where sites with higher densities were generally located in leeward areas, with less wave energy. Further studies would be required to determine how this factor may affect the population dynamics of this important herbivore across a wave energy gradient.

One relatively worrisome finding was the low abundance of small size individuals, and points toward recruitment limitation as an explanation for the slow recovery of *D. antillarum*. An evaluation of this particular factor may be driven by conducting long-term studies that includes capture-mark-recapture techniques. We tested several tagging techniques in *D. antillarum* with disappointing results (see Chapter III). We did not find any of these tags suitable due to their low retention or high mortality. Some tags induced high autotomy, low retention, and also negative effects on survival. The lack of successful tagging methods was substituted with the development of a demographic model based on size-classes (see Chapter IV). Results of this model indicate relatively high adult survival, and low stasis but high growth transition in the small individuals. Elasticity analysis indicates that survival of large sized echinoids can potentially contribute most to changes in growth rate for all sites. Recruitment rate was low and

exhibited high spatial and temporal variability, which agrees with the findings in Chapter I. Numerical projections of the populations indicated that no site would exhibit an increase in density under current recruitment rates, but doubling recruitment would result in densities above 1 urchin.m⁻². In summary, local recovery will require spatial and temporal co-occurrence of high recruitment and survival rates. Further studies will have to determine what factors affects the recruitment process, and how current environmental conditions impact the reproductive success of *D. antillarum*.

The dominance of large sea urchins and the scarcity of small ones suggested the importance of predation analysis in the dynamic of *D. antillarum*. Our experimental removal of two common wrasses demonstrated that these fishes can significantly increase the relative abundance of small and medium-sized sea urchins and modify *Diadema* population structure. Although conclusions cannot be drawn from only 1 yr of data, population structural changes were not seen in the study site (see Chapter I). This suggests that one year is enough time to detect differences in size-class structure. However, changes in density of the most abundant size class (i.e. the large urchins), should take more time to manifest.

The omnivorous behavior displayed by *D. antillarum*, which was confirmed in this study, suggests the high plasticity of *Diadema* in the use of multiple food resources depending on environmental availability. This generalist feeding behavior may be considered an advantageous characteristic, and casts doubt on the idea that food resources might be a limiting factor in the species recovery process. Nevertheless, further studies should include invertebrate samples to clarify and quantify the real contribution of all potential food resources in the diet of *D. antillarum*. Other recommendation may include the

development of fixed permanent sites throughout Puerto Rico and Culebra, where larvae availability and predator abundance, including small fish predators, are measured systematically as part of a concerted management effort.

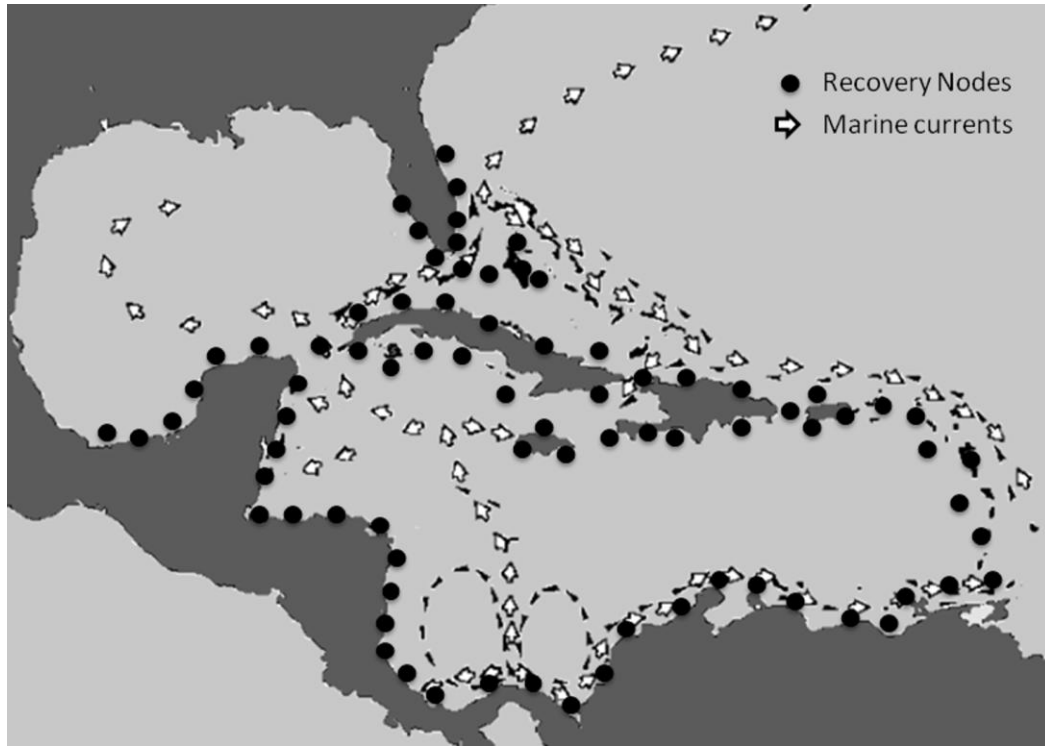


Figure 1. The Recovery Nodes System to strengthen the larvae flows in the Caribbean. Black dots represent the hypothetical node. White arrows represent the regional marine current pattern.

The existence of genetic structuring among populations of the Caribbean (Lessios 1985), in addition to the pathway followed during the mass-mortality event (Lessios *et al.* 1984) suggest the existence of a metapopulation where a constant flux of larvae maintains regionally interconnected populations. This Larvae Transporting Belt (L.T.B.) lost its capacity to maintain high-density populations due to a larvae deficit caused by the demographic crash. Three decades later, larvae supply seems to be a critical factor hindering the recovery of *D. antillarum* (see Chapter IV). If the above is true, what is

needed for a region-wide recovery is a regional action plan to strengthen the L.B.T (Fig. 1). A primary objective of this regional effort should include a successful larvae marking technique to determine local larval dispersal range. Once known, we would establish high-density populations of approximately 6 ind m⁻². These artificial-enhanced nodes, called Recovery Nodes (R.N.) would be located at each island of the Caribbean, and the number of R.N. would depend on island total area, and local larvae dispersal range. High-density nodes would increase the successful fertilization rate, enhancing the Caribbean L.B.T., and promoting more arrival of larvae to the surrounding populations. Marine current patterns will be necessary to determine what areas have the major probabilities to receive those larvae fluxes from nodes. This will help to measure the impact of the management strategy and how the nodes can contribute to the recovery process of *D. antillarum*.



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Appendix A1 Annual transition and mortality matrices of the sea urchin *Diadema antillarum* in four sites of Puerto Rico. Size-independent distribution was assumed for mortality (see methods for details. Mortality rates (μ) are given in deaths.year⁻¹.

Site	QP matrix				μ matrix			
		S	M	L		S	M	L
CGD	Small	0.014	0	0		0.001	0	0
	Medium	0.987	0.906	0		0.081	0.075	0
	Large	0	0.094	0.753		0	0.008	0.082
μ 0.247								
LQY	S	0.519	0	0		0.019	0	0
	M	0.482	0.859	0		0.017	0.031	0
	L	0	0.142	0.892		0	0.005	0.036
μ 0.108								
TMD	S	0.656	0	0		0.039	0	0
	M	0.344	0.847	0		0.020	0.050	0
	L	0	0.153	0.822		0	0.009	0.059
μ 0.178								
MLN	S	0.462	0	0		0.021	0	0
	M	0.539	0.738	0		0.024	0.033	0
	L	0	0.262	0.864		0	0.012	0.045
μ 0.136								