Population Status of the Black Sea Urchin *Diadema antillarum* (Philippi) in La Parguera, Puerto Rico, 20 Years After the Mass Mortality Event.

By

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Abstract

Before the 1983 mass mortality event, the conspicuous sea urchin *Diadema antillarum* was considered a key species in Caribbean coral reefs. This epizootic event caused mortalities of up to 99% in many localities of the western Atlantic and Caribbean. In this study, the current status (after 20 years) of populations of *D. antillarum* was assessed in five reef localities off the southwest coast of Puerto Rico. Densities were estimated by counting all urchins in each of five band transects (20 x 2 m) in each of three depth intervals (0-3 m, 4-10 m, >10 m) at each reef site. Size structure of the urchin populations were estimated by measurement of maximum test diameter of every urchin found along each band transect. Habitat complexity (i.e., rugosity) was determined using the chain method of CARICOMP. Density differences were compared as a function of site, rugosity and depth. A linear regression showed that the densities of *D. antillarum* where influenced by factors like depth and site ($P<0.0001$). The test revealed that in the present state of urchin populations, rugosity did not have a statistically significant effect on densities but a trend to higher densities in more complex areas was observed. Mean densities per reef varied between 0.01-0.82 ind/m², with a mean density of 0.23 ind/m² for the entire area surveyed. A significant decrease in densities of *D. antillarum* with depth was found. Size distributions varied between depth intervals (K-S, $P<0.05$) and amongst reefs in some occasions, with higher densities of medium-big urchins in shallow waters and higher densities of small individuals in deeper areas. Reproductive dynamics was assessed using the gonad index (GI= 100 x weight of the gonad divided by the weight of the urchin). Urchins were dissected every month for a period of fourteen months. An increase in GI indicates active gametogenesis and a decrease that urchins spawned. *D. antillarum* in La Parguera reproduced all yearlong with a reproductive peak between the Spring and Summer in 2001. A correlation between sea surface temperature and GI indicates a possible influence of this factor on the reproductive biology of these urchins. GI started to increase in the fall and the highest proportion of spawning urchins and decreasing GI was observed right after the sea surface temperature began to increase in the Spring following the winter lows. Minimum reproductive size (smallest sexually mature individual) was 3.8 cm in diameter. Sex ratio (♂/♀) was 0.89:1. Overall, it appears that *D. antillarum* are slowly coming back and populations show high variability in their distribution, densities and size structures within and across different reef localities.

Resumen

El erizo negro *Diadema antillarum* era considerado como una especie "clave" en los arrecifes del Caribe antes de la mortandad masiva de 1983. Este evento epizoótico causó mortandades de hasta 99% en muchas localidades del Caribe y Atlántco oeste. En este estudio se determinó el estado actual (veinte años despues) de las poblaciones de *D. antillarum* en cinco localidades en la costa suroeste de Puerto Rico. Las densidades fueron estimadas contando todos los erizos en cada uno de cinco transectos de banda (20 x 2m) en cada uno de tres intervalos de profundidad (0-3m, 4-10m, >10m) dentro de cada arrecife. Se midió el diámetro de caparazón de todos los erizos dentro de cada transecto para determinar la distribución de tamaños de la población. La complejidad (rugosidad) del sustrato se estimó usando el método de la cadena de CARICOMP. Se analizaron las diferencias en densidades y tamaño de *D. antillarum* en función de localidad, profundidad y rugosidad. Regresión lineal reveló que las densidades de *D. antillarum* son afectadas por factores como profundidad y localidad (P<0.0001). No se encontró una correlación significativa entre las densidades y la rugosidad pero los resutados indican una tendencia del aumento en densidades con un incremento de la complejidad del sustrato. Las densidades promedio por localidad variaron entre 0.01-0.82 ind/m² con una densidad media de 0.23 indv/m² para el área muestreada. Las densidades de *D. antillarum* disminuyeron significativamente con profundidad con la mayoría de los erizos poblando las áreas someras. El tamaño promedio también varió con la profundidad (K-S, P<0.05) con una proporción mayor de erizos pequeños en aguas profundas y erizos medianos y grandes en areas someras. Para verificar el patrón reproductivo de *D. antillarum* se utilizó el índice gonadal (IG = 100 x peso de la gónada entre peso del erizo) y preparaciones histológicas de cortes de gónadas. Un aumento en el IG indica una gametogenesis activa, una reducción indica que hubo desove. Los erizos se reprodujeron todo el año con un pico reproductivo entre primavera y verano para el 2001. Se encontró una correlación positiva y significativa entre el IG y la temperatura, indicando el posible efecto de este factor en el ciclo reproductivo de este erizo. El tamaño mínimo reproductivo fue estimado en 3.8 cm de diámetro y las poblaciones mostraron una proporción de sexos (♂/♀) de 0.89:1. En general, las poblaciones de *D. antillarum* muestran una lenta recuperación en el área de La Parguera. La poblaciones actuales muestran una alta variabilidad en su distribución, densidades y estructuras de tamaño dentro da cada arrecife y entre arrecifes.

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I. Introduction

The conspicuous black sea urchin *Diadema antillarum* Philippi was considered as a keystone species in Caribbean coral reef ecosystems before the mass mortality event of the early 1980's (Lessios et al. 1984a). Before 1983, this urchin had a wide geographic distribution throughout the wider Caribbean and the western Atlantic, from Bermuda to as far south as Surinam (Bauer 1980). In some localities, densities of up to 71 ind/m² where recorded (Hunter 1977, Weil 1980, Lessios et al. 1984, Weil et al. 1984, Sammarco 1982). The high number of urchins in some localities could have been the result of high reproductive success and low number of predators (Odgen et al. 1973, Weil et al. 1984). In the Caribbean, this urchin was present in most shallow water habitats including coral reefs, sea grass beds, rocky shores and sandy bottoms. Populations had a variable distribution of densities and size structure with respect to the depth gradients and could be found as deep as 40 m (Randall et al. 1964, Lessios et al. 1984a, Weil 1980, Weil et al. 1984).

The high densities of *D. antillarum* and their feeding habits played an important role on the structural dynamics of coral reef communities. *D. antillarum* is an omnivorous species with a preference for algae (Weil, 1980). The pressure exerted by the urchin on algal communities helped to maintain a high diversity and a low biomass of algae (Randall et al. 1964, Ogden et al. 1973, Sammarco et al. 1974, Carpenter 1981, 1985, 1990). In addition to their herbivorous nature, the urchin caused high rates of bioerosion, re-processing and bioeroding up to 97 tons of calcium carbonate per hectare per year in some localities (Table 1) (Hunter 1977, Stearn & Scoffin 1977, Weil 1980).

Table 1: Yearly bioerosion rates (kg/m²/yr) reported for some species of sea urchins.

Species	Bioerosion (kg/m²/yr)	Source
Diadema antillarum	5.68-9.00	Hunter, 1977; Weil, 1980
Diadema savignyi	3.87	Bak, 1990
Diadema mexicanum	5-19	Glynn, 1988
Echinotrix diadema	0.796	Bak, 1990
Eucidares thouarsii	27.1	Glynn, 1988
Echinometra mathaei	0.372-8.3	Bak, 1990; Conand et al., 1997

The feeding habits of *D. antillarum* also had an effect on the recruitment of juvenile corals by preserving a high availability of substrate for colonization. Because of the indiscriminate grazing by *D. antillarum,* damage was also inflicted to recruits and coral tissue of adult coral colonies (Sammarco 1982; Weil 1980, Weil et al. 1984).

A massive die off of *D. antillarum* was reported during 1983-84 all over the western Atlantic and the Caribbean. The die off was first reported in Galeta Point, Panamá in January of 1983**.** The disease followed the direction and time of the ocean currents, dispersing all over the Caribbean, Gulf of Mexico, and even up to Bermuda in the middle of September 1983 (Lessios et al. 1984b). The disease covered an area of 35 million square kilometers killing up to 99% of *D. antillarum* in some locations (Table 2) (Lessios et al. 1984b, Bak et al. 1984, Hughes et al 1985, Hunte et al. 1986, Lessios 1988, Hunte & Younglao 1988). This die off has been the most documented mass mortality of any marine invertebrate (Pearse et al. 1977), including the major events involving sea

urchins in California and Canada (Pearse 1977, Miller et al. 1983, Sheibling et al. 1984) and sea stars in the Gulf of California (Dungan 1982).

The apparent cause of the mortality was a water-borne pathogen because the die-off showed a dispersion pattern similar to the surface currents of the Caribbean and western Atlantic (Bak et al.1984, Lessios et al. 1984a,b, Hughes et al. 1985). Populations of *D. antillarum* in aquariums that had the water pumped from nearby sea waters, like Discovery Bay in Jamaica and the Coral World in St. Thomas, died at the same time as the free living populations in near by reefs (Lessios et al., 1984). Curiously, the pathogen was never identified. Three arguments support the hypothesis that the causative agent

Table 2: Densities of *Diadema antillarum* (ind/m²) reported before and after the 1983 mass die off for some regions in the Western Atlantic and Caribbean.

Site	Reef	Ind/m² before die off	Ind/m² after die off	Source
Barbados	North Bellairs	17.3	1.8	Hunte et al., 1988
	Sandridge	14.1	2.9	Hunte et al., 1988
	Golden Palms	12.9	3.8	Hunte et al., 1988
Jamaica	Montego Bay	6.3	1.8	Hughes et al., 1985
	Rio Bueno	7.6	0.5	Hughes et al., 1985
Curacao	H. Beach	3.97	0.01	Bak et al., 1984
	Buoy I	2.93	0.01	Bak et al., 1984
	Buoy III	4.16	0.05	Bak et al., 1984
Puerto Rico	Jobos Bay	2.6	?	Rivera et al., 1976
	San Juan	13.8	?	Bauer, 1980
	Guayanilla	3.0	0.13	Vicente et al., 1985
	Turrumote	12.7	?	Craft, 1975
Panama	San Blas	3.63	0.24	Lessios, 1988

was a water borne, highly specific pathogen and not a pollutant; (1) the extent of dispersion that the disease had without decreasing its virulence. It did not affect the other six species of urchins present in the same areas. Normally a pollutant does not stay toxic in an area so large affecting only one species (Bak et al. 1984, Lessios et al. 1984); (2) in Panama, where the die off began, there were no sudden changes in water salinity or temperature during the die off (Lessios et al. 1984); and (3) there were reports of loss of zooxanthellae and death of some corals in some areas of the Caribbean, but the phenomenon did not follow the spatial and temporal sequences of the D. antillarum die off (Lessios et al. 1984).

In Puerto Rico, diseased urchins were first reported in January of 1984 in Laurel reef (Vicente et al. 1984). Quantitative estimates of *D. antillarum* densities in the intertidal, high wave action, broken rock zone on Condado Beach at the northeast part of Puerto Rico, showed average densities of 13.8 ind/m² before the die off (Bauer 1980). Other quantitative observations before the die off indicated average densities of 3 ind/m² at 1 m depths and 1 ind/m² in shallower *T. testudinum* beds. Jobos Bay, on the southeast coast, had mean densities of 2.6 ± 1.3 ind/m² in *T. testudinum* beds with clumps of up to 17-19 ind/m², and mean densities of 12.9 ind/m² urchins were present on reefs before the die off (Vicente et al. 1982). In Jobos Bay, Puerto Rico, the intense grazing of *D. antillarum* controlled the depth distribution of *T. testudinum* sea grass (Vicente et al. 1982). In areas where few *D. antillarum* were found the presence of coral colonies was low (1.25/m² to 0/m²) (Rivera 1976).

In La Parguera, on the south-west coast, *D. antillarum* was more abundant on outer reef localities than on inner reefs because the slope was slight and because there

were extensive areas of *Acropora palmata*, *A. cervicornis* and dead coral rubble which were good habitats for urchins (Craft 1975). Additional mortalities of other sea urchins species have been reported for different localities in Puerto Rico. The species affected were *Astropyga magnifica*, *Eucidaris tribuloides* and *Tripneustes ventricosus* (Williams et al. 1986, 1996).

The mass mortality of *D. antillarum* brought about many changes in coral reef communities. In places where over-fishing of other herbivores took place, the lack of *D. antillarum* was especially important (Hay 1981, 1984, Carpenter 1990). There was a significant change in abundance, structure and diversity of coral reef algae. Green and red encrusting algae decreased in abundance due to displacement and overgrowth by other macro-algae (Sammarco 1982, Carpenter 1990). Filamentous algae and algal turfs were displaced by macro-algae such as *Laurencia obtusa*, *Jania coralina*, *Sphacelaria sp.*, *Dyctiotales*, *Amphiroa* y *Gelidiela*. Near the island of Jamaica, the percent cover of non-crustose algae increased from 30.7% to 49.7% during a two-week period and after four months, the percent cover had increased to 72.3% (Lidell 1986). There was a significant increase in algal biomass in almost every shallow reef (Carpenter 1985). As a consequence, increased shading, competition for space and the disappearance of bare areas for coral recruitment by the algae led to a decrease in coral cover and diversity (Carpenter 1985, Lidell 1986). Rates of bioerosion and sediment production decreased significantly over the distributional range of the urchins (Hunte et al. 1986, Weil, pers, comm.).

Information on the recovery of *D. antillarum* in various localities in the Caribbean has been recently reported (Edmunds et al. 2001, Moses et al. 2001, Chiappone et al.

2002). In La Parguera, observations of significant numbers of D. antillarum in some localities were recently reported (Williams et al. 1986, Torres et al. 2001, Weil et al. in press). Recent reports did not show a significant increase in densities of *D. antillarum* in many localities around the Caribbean even after two decades of the die-off (Table 3) (Lessios 1988, Karlson & Levitan 1990, Cho et al. 2000, Edmunds et al. 2001, Moses et al. 2001, Torres et al. 2001, Chiappone et al. 2002, Weil et al. in review).

Every study of the population dynamics of an organism requires basic information of its reproductive biology. This is important when natural populations have suffered mass mortalities and/or drastic reductions in densities (bottle necks) with possible long-term evolutionary consequences (Lessios 1986). The gonad index is a measure of the relationship between weight of the gonads and total weight of the organism. This index is used to characterize the reproductive cycle of many marine species. For sea urchins like *Lythechinus variegatus* the gonad index varied between years, but the spawning period and gametogenetic cycle did not vary much within years (Fiji 1960, Moore et al. 1972). By contrast, the sea urchin *Tripneustes esculentus* in Florida had a significant variation in its spawning period in three years maybe due to changes in environmental conditions (Moore et al. 1963b). These processes can vary to a high degree because of changes in temperature and salinity in different localities (Moore et al. 1972, Pearse 1974). The gonad index of urchins varies during the different lunar cycles. In some cases, the index increases during the new moon when they are ready to spawn and decrease the following week after spawning (Bauer 1976).

In the case of *D. antillarum*, early studies have shown that the spawning season of the urchin varies with locality and with lunar phases (Table 4) (Iliffe et al. 1982). As an

example, for *D. antillarum* populations present in the Canary Islands, the spawning

season coincides with the period of most rapid temperature rise (June-July), but the new

reproductive cycle is initiated as temperature continues to increase (Garrido et al. 2000).

Historical data describing the reproductive characteristics of *D. antillarum* in Puerto Rico

are not available. Only qualitative observations showed spawning of *D. antillarum* during

October of La Parguera (Rivera et al. 1976). In this locality other sea urchin species

Table 3. Recent densities (ind/m²) of *Diadema antillarum* reported for some regions in
the Western Atlantic and Caribbean.

Site	Name of reef	Indv/m² of *D. antillarum*	Source
Florida	Looe Key	0.013	Chiappone et al., 2002
Florida	American Shoal	0.01	Chiappone et al., 2002
Jamaica	Discovery Bay	0.0	Haley et al., 2001
Jamaica	Discovery Bay	0.02	Haley et al., 2001
Jamaica	Discovery Bay	0.1	Cho et al., 2000
Jamaica	Discovery Bay	0.6	Cho et al., 2000
Puerto Rico	La Parguera	0.83-1.39	Weil et al. (in press)

Table 4: Months of reproductive activity reported for *Diadema antillarum* in some
regions around the Western Atlantic and Caribbean**.**

Site	Season of spawning/ Peak	Source
US Virgin Islands	All year/winter & early spring	Randall et al., 1964
Panamá	All year/October	Lessios, 1981
Barbados	January to April/ April & March	Lewis, 1996
Florida Keys	October to December/November	Bauer, 1976
Curacao	All year/ January & February	Iliffe and Pearse, 1982

showed an increase in the gonad index during summer and fall when the water

temperature is high (Cameron 1986). There is no information, however, on the general

aspects of the reproductive biology such as gametogenesis, sex ratios, frequency of spawning and minimum reproductive size for Puerto Rico**.**

The first objective of this project was to assess the present status of the populations of *D. antillarum* in five reef localities off La Parguera, Puerto Rico, 20 years after the die-off. Urchin abundance and size structure were assessed in each site and their variability was assessed between depth intervals within each reef, and across reef sites. As an additional objective of this project, a brief characterization of the reproductive biology of *D. antillarum* was completed.

The questions and hypotheses that were addressed in this study were:

Q1- Have populations of *D. antillarum* recovered since the 1983 mass mortality event?

H11- Populations of *D. antillarum* have recovered after 20 years of the massive die off, their densities are similar to pre-mass mortality densities.

Q2- Are densities and size distribution of *D. antillarum* influenced by the depth gradient and by site?

H21- Depth and site do not influence the size distribution and densities of *D. antillarum.*

Q3- Do the populations of *D. antillarum* have a reproductive peak during the year?

H31- Populations of *D. antillarum* do not have a yearly reproductive peak, they reproduce all year-long.

Q4- What is the sex ratio (male/female) for populations of *D. antillarum* in La Parguera after the mass mortality?

H41- The sex ratio of *D. antillarum* in La Parguera is different from the normal 1:1 ratio.

Q5- What is the minimum reproductive size of *D. antillarum* populations in La Parguera?

H51- Urchins minimum reproductive size is 5 cm of test diameter.

II. Materials and Methods

II-1. Study Area:

Five reefs were selected for quantitative studies in La Parguera Natural Reserve off the southwest coast of Puerto Rico. To facilitate logistics all of the sites were located near Isla Magueyes, the field station of the Department of Marine Sciences, University of Puerto Rico. The selected reefs were: Turrumote (17° 56.07' N - 67° 01.2' W), Mata la Gata (17° 57.640. 67° 02.165), Cayo Enrique (17° 57.3' N - 67° 02.8' W), Laurel (17° 56.501 N -67° 03.296 W) and Media Luna (17° 56.283' N - 67° 0.2457' W) (Fig 1). All of these reefs are part of a larger group of patch and fringing reefs that surrounds and protect the coastal community of La Parguera from open Caribbean waters. The sites were characteristic fringing reefs on the shallow insular shelf located 1 to 2 km off shore. Each locality is about 1 km long and reefs lie on a NE-SW orientation with a maximum depth of 20 meters (Cameron 1986). These reef sites follow an east-west direction with Turrumote, the one further east, as the most exposed to wave action. Media Luna and Laurel are consecutively further west and less exposed to wave action. Cayo Enrique and Mata la Gata are found between Media Luna and Turrumote and are the closest to the coast and less exposed to high seas. A recent study assessing the population densities and size structure of *D. antillarum* in three other reef areas and three sea-grass mounds within La Parguera (Weil et al. in press) was conducted two years before this study.
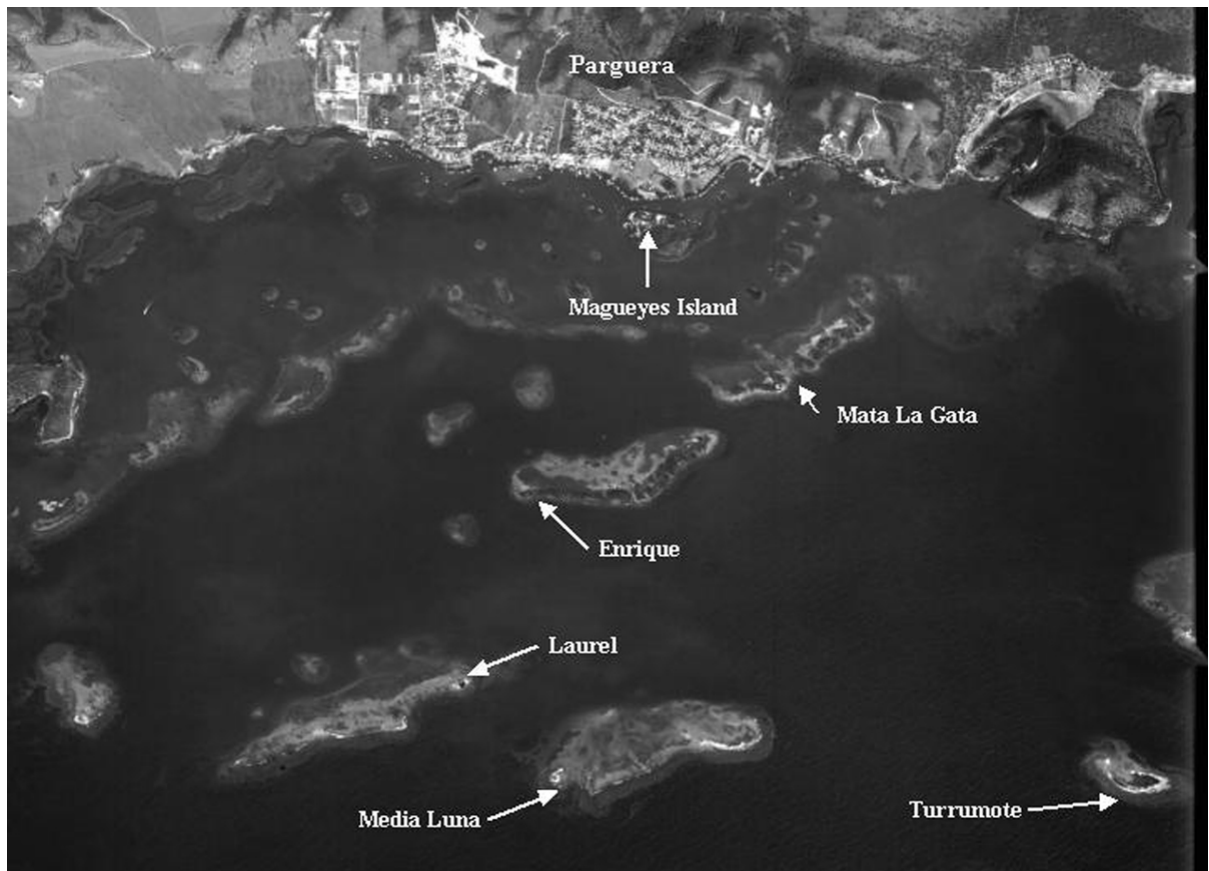
Figure 1. Map of the La Parguera, Puerto Rico showing the sampling localities. The five reef sites surveyed (Turrumote, Media Luna, Laurel, Cayo Enrique and Mata la Gata) are in an East-West and North-South orientation. Mata la Gata and Enrique are semi-protected reef compared to Laurel, Turrumote and Media Luna.

II-2. Density and size structure of the black sea urchin *Diadema antillarum*:

The band transect method was used to estimate the densities of *D. antillarum* in each reef site selected for the study. Each transect was 20 m long x 2 m wide for a total area of 40 m² (Weil et al. 1984). Transects were extended parallel along the fore reef originating from randomly selected points on each site. Each reef was divided in three depth zones: 0-3 m, 4-10 m and greater than 10 m. These three depth intervals were selected to test the hypotheses that *D. antillaurm* densities were similar along the depth gradient and in reef areas with different rugosity. Five band transects were placed at each depth interval (200 m² per depth zone) for a total of 15 transects (600 m²) per reef site. Band transects were anchored on both ends by permanent re-bars hammered into the substrate. Both scuba and free diving were used to conduct the surveys. A diver swam systematically along the band-transect area, checking all crevices and counting all the urchins found within the band transect boundaries. After the density counts were conducted, every urchin found inside the transect area was collected and measured. Special care was taken to avoid missing small urchins hiding in the crevices of the reef. A long stick and salad tongs where used to remove and grab the urchins from within the crevices. A two-point compass was superimposed on the oral cavity side of the urchin avoiding protuberances and then compared to measuring tape (1 mm resolution) glued to the side of the slate used to collect data (Weil et al. 1984). Counts were conducted only during early morning daylight hours to avoid the effect of migrations in and out of crevices and depth intervals of *D. antillarum* during early morning.

II-3. Rugosity:

Rugosity is an index that roughly estimates the complexity or "wrinkling" of the substrate of the area sampled at each site. Areas of higher structural complexity may offer more protection and cover for the urchins. A 10 meter long chain was extended along each 20 m belt transect area and was carefully draped over the reef surface until the entire distance between the two points in a straight line was covered. The rugosity index was determined dividing the total length of the belt transect (L1=20 m) by the total length of the chain (L2). Rugosity = [Total length of transect (20 m)]/ [Total length of wrapped around the bottom profile (m)]. These indexes are raw estimates of structural complexity of the area sampled. If the index is near or equal to 1, the substrate is nearly flat and less complex. The lower the index the more complex and irregular the substrate.

II-4. Reproductive Biology of the sea urchin *Diadema antillarum*:

One of the objectives of this study was to assess current characteristics of the reproductive biology of *D. antillarum* and compare it with the information available before the mass mortality. The annual reproductive cycle was assessed using the gonad index (GI), an estimation (%) of the reproductive potential of sea urchins (Iliffe et al. 1982). Because of high abundances of medium to large urchins at the study sites and to insure sexual maturity of the samples, the gonad index was determined only for urchins between 6 and 8 cm in diameter collected each month for fourteen consecutive months. The number of samples collected for each month fluctuated between 25 to 35 urchins. All urchins were collected from the fore reef area of the Laurel and Mata la Gata reefs during the quarter moon phase, which occurs during the week before the new moon, a time when

D. antillarum are most likely to spawn (Bauer 1976, Iliffe et al. 1982). The urchins were collected using a stick and large salad tongs, put into plastic baskets, and transported to the laboratory for processing**.** In the laboratory, the urchins where kept alive in a tank with running seawater until processing. Gathering samples during the same lunar phase, avoided confounding effects from the spawning lunar rhythm of the urchin (Bauer 1976). Each urchin was weighed to the nearest 0.1g using a digital balance (Mettler − OHAUS Explorer 0.01g). The animals were carefully opened using surgical scissors to cut around the peristome on the underside of the urchin. The Aristotle's lantern, gut, and then gonads were carefully removed, cleaned and weighed to the nearest 0.1g, photographed and preserved in a 5% formalin solution (Fig 2). The gonad index was calculated by dividing the weight of the gonad by the total weight of the urchin and then multiplied by 100 to obtain a percentage value (Scheibling & Meidel 1998). The average gonad index per month was used to characterize the annual reproductive cycle of the sea urchin.

II-5. Sex Ratio of *Diadema antillarum* populations.

The sex ratio of populations of *D. antillarum* was determined by examining the prepared slides of gonad tissue, including the gonad tissue used to determine the GI. Each specimen was sexed by checking thin slides of their gonadal tissue using a digital camera attached to a compound microscope (Olympus Z40). After extracting the gonads, they were fixed in a 5 % formalin solution for a period of four hours. The 5 % buffered formalin was prepared by diluting 90 ml of salt water in 10 ml of 100% formalin. Distilled water was not used to avoid the breakage of tissue due to the unbalanced osmotic pressure between the solution and the tissue sample. Fixed samples were then
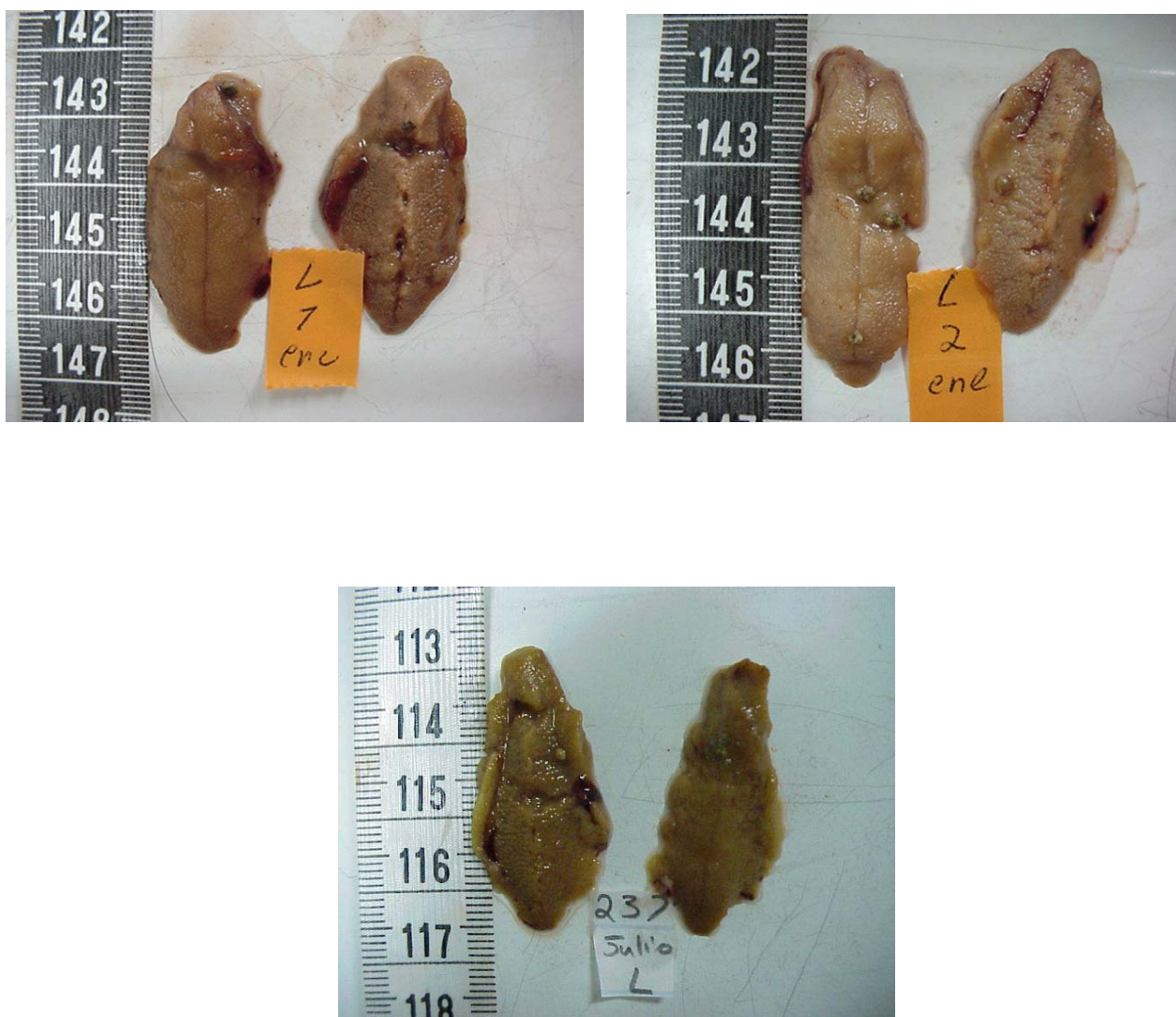
Figure 2. Photos of *Diadema antillarum* gonads after dissection and removal from urchins.

preserved in a plastic container using a 70 % ethanol that was prepared by mixing 750 ml of 95 % ethanol with 250 ml of distilled water.

After the samples were preserved, slides of the urchin gonad tissue were prepared using standard histological techniques to be analyzed later for differentiation of sexes of the different urchins (See Appendix 1). Once the tissue slides were prepared, careful examination under a light microscope was done to distinguish male from female gonads. For this study, reference slides of *D. antillarum* were not available to be use as reference. Instead, photos of the Nova Scotia green sea urchin *Strongylocentrotus droebachiensis* gonad tissue sections published by Meidel et al. (1998) were use as reference. In addition, photos of gonad sections for *Lytechinus variegatus* by Moore et al. 1963 showed how an urchin eggs and a spermatozoans look like.

II-6. Minimum reproductive size of the sea urchin *Diadema antillarum*.

Small urchins (< 3 cm) were collected from Laurel and Mata la Gata each month, at the same time the bigger urchins were collected. There were months that no small urchins were collected due to their low abundance. These urchins were collected to determine the minimum reproductive size of *Diadema antillarum* for La Parguera. The minimum reproductive size was determined based in the assumption that any small urchin that showed presence of visible developed gonads was sexually mature and the urchins without visible gonads were considered sexually immature. No tissue analysis was done for the gonads found on the small urchins.

II-7. Statistical Analysis:

To test if densities of *D. antillarum* were similar between depths within a reef and between the reefs surveyed in La Parguera, a One Way ANOVA was initially used. However, because the data did not meet the conditions for the parametric test, a non-parametric Kruskal-Wallis test was used to examine for differences in mean densities. A Dunn's multiple comparison test was used a posteriori to asses significant differences in densities between sites and depth intervals (Sokal & Rohlf 1981). The size frequency distribution of the urchins by depth interval between the different sites was compared using a Kolmorogov-Smirnov (K-S) test.

A regression analysis was used to assess the relationships/effects of depth, rugosity and site on mean densities and size of *D. antillarum* in each locality and in the area. The distribution pattern of the urchin populations throughout the front reef was determined for each of the depth intervals using the variance to mean ratio (Sokal & Rohlf 1981). If the ratio was higher than 1 it showed that the urchin distribution was aggregated, equal to 1 was random distribution and less than one was even distribution.

III. Results

III-1. Population Densities of *Diadema antillarum* in La Parguera, Puerto Rico.

The sea urchin *D. antillarum* was present in all the localities surveyed and many others visited throughout La Parguera. The median value for urchin density (ind/m²) (range) found for the entire area surveyed (75 transects) was 0.08 ind/m² (0.00-0.82) (Table 5). Overall, this low average density is significantly lower than pre-mortality densities indicating that populations of *D. antillarum* have not fully recovered; therefore, rejecting the first null hypothesis (H_{01} = *Populations of D. antillarum have recovered after 20 years of the massive die off*).

Of all the sites sampled, Mata la Gata had the highest mean urchin density (0.13 ind/m²), followed by Laurel (0.0.03 ind/m²), Media Luna (0.15 ind/m²), Cayo Enrique (0.00 ind/m²) and finally, Turrumote with the lowest density (0.00 ind/m²). A linear regression showed that densities of *D. antillarum* were significantly dependent on site (P<0.001). Turrumote had a significantly (P<0.05, Kruskal-Wallis) lower mean urchin density compared to the other four reefs. Media Luna had significantly (P<0.05, Kruskal-Wallis) higher densities compared to Cayo Enrique and Turrumote. Mata la Gata, Laurel and Cayo Enrique had similar urchin densities, therefore, rejecting one of the second null hypotheses (H_{02} = *site do not influence the densities of D. antillarum*).

Urchin densities across depth intervals varied (Fig 3) significantly (Kruskal-Wallis, P<0.05) with higher densities in the shallow (0.30 ind/m²) and intermediate (0.03 ind/m²) areas compared to the deepest interval (0.00 ind/m²).

Table 5. Median values and ranges of values for densities (ind/m²), as well as size (cm) (± SD) of *Diadema antillarum* for each reef site and depth zone surveyed near La Parguera, Puerto Rico during the year 2001.

Reef Site	Depth Zone (m)	Median ind/m² (Range)	Site Median ind/m² (Range)	Mean Size (cm)/SD	Site Average (cm)/SD
Turrumote	0-3 m	0		0	
	3.1-10 m	0.03 (0.00-0.50)	0.00 (0.00-0.50)	7.4 (1.2)	6.55 (2.38)
	> 10.1 m	0.00 (0.00-0.03)		2.2	
La Gata	0-3 m	0.35 (0.00-1.10)		6.8 (2.0)	
	3.1-10 m	0.85 (0.00-1.10)	0.13 (0.00-1.10)	6.4 (1.9)	6.51 (1.99)
	> 10.1 m	0.00 (0.00-0.08)		1.2 (0.4)	
Cayo Enrique	0-3 m	0.50 (0.15-1.40)	0.00 (0.00-1.40)	7.1(1.3)	7.09 (1.34)
	3.1-10 m	0		0	
	> 10.1 m	0		0	
Laurel	0-3 m	0.78 (0.28-1.48)		6.9 (1.4)	
	3.1-10 m	0.03 (0.00-0.30)	0.03 (0.00-1.48)	5.9 (2.0)	6.84 (1.48)
	> 10.1 m	0		0	
M.Luna	0-3 m	0.28 (0.15-0.50)		7.0 (1.5)	
	3.1-10 m	0.15 (0.03-0.48)	0.15 (0.03-0.50)	6.2 (2.0)	6.56 (1.74)
	> 10.1 m	0.05 (0.03-0.20)		6.7 (1.3)	

A multiple comparison test showed significant differences in densities of *D. antillarum* when compared between the three depths intervals within each reef (Dunn's test, $P<0.05$), therefore one of the second null hypothesis was also rejected (H_{02} = urchin densities were not influenced by depth). Almost all reefs showed the highest densities of *D. antillarum* in the shallow fore reef zone (0-3 m) (Fig. 3). The pattern of decreasing densities with increasing depth was evident in almost all of the sites except Turrumote and Mata la Gata where higher densities were observed in the middle depth zone. In Turrumote, no urchins were found in shallow waters, with 0.03 ind/m² urchins found at

the intermediate zone and only one individual recorded in deep waters (> 10 m) (Fig 3A).

At Mata la Gata, the highest density of *D. antillarum* was recorded at the middle depth

zone (0.85 ind/m²) followed closely by the shallow depth zone (0.35 ind/m²) and only

two urchins found in deep waters (Fig 3B). The decrease in densities across the different

depth zones in Media Luna changed less abruptly than at the other reef sites. Media Luna

reef had the highest abundance of *D. antillarum* in the shallow fore reef zone (0.28

ind/m²) and showed the highest density of *D. antillarum* (0.05 ind/m²) in deep waters of

all the reefs sampled (Fig 3C). Mean population densities where found to be more than

one order of magnitude higher in the shallow fore reef zone at Laurel (0.78 ind/m²) and

Cayo Enrique reef (0.50 ind/m²) than in deeper waters where no urchins were found (Fig

3D & 3E). A Kruskal-Wallis test confirmed that depth had a significant effect (P<0.05)

on the distribution (densities) of *D. antillarum*.

The variance to mean ratio (S²/X) showed that *D. antillarum* had an aggregated

spatial distribution around the surveyed area. The variance to mean ratio on each reef for
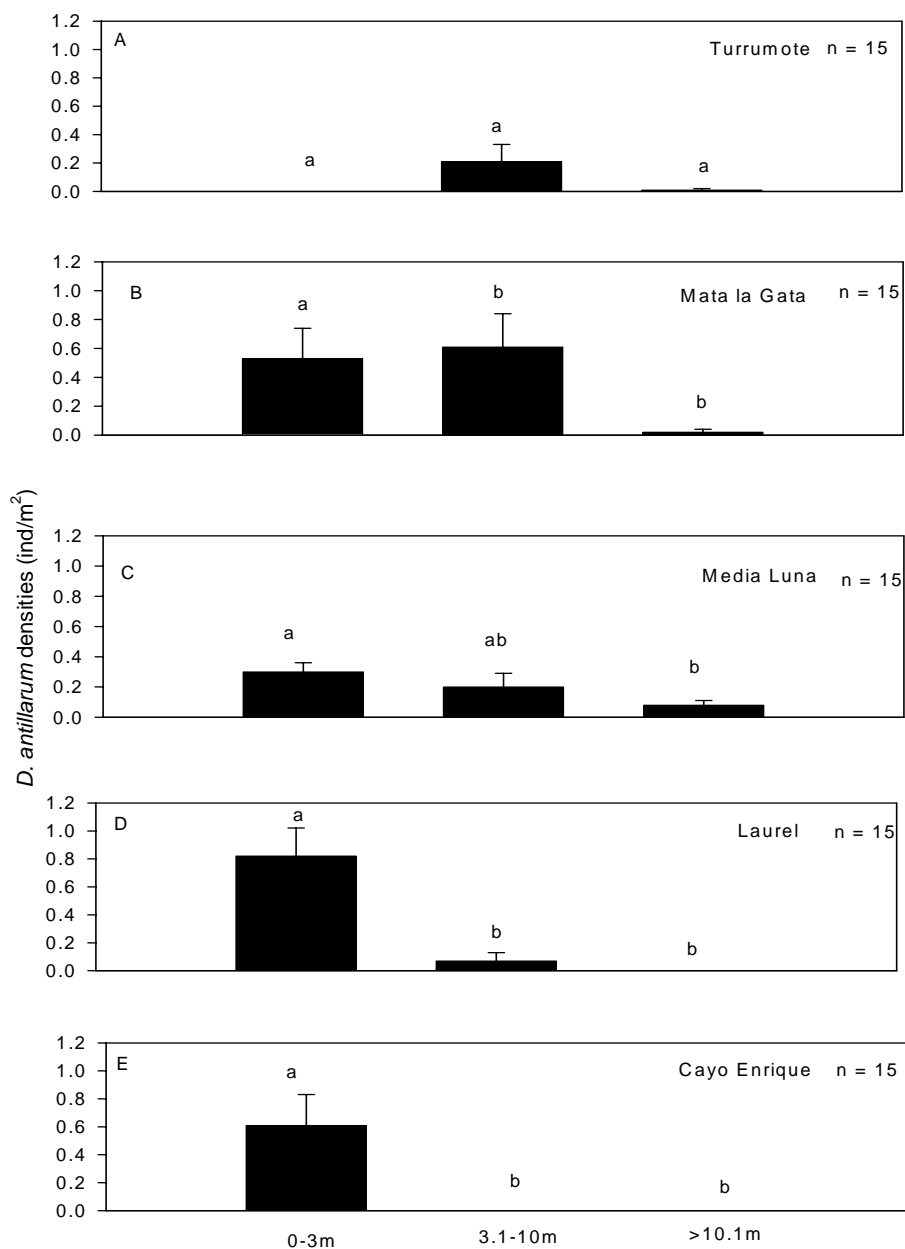
Figure 3. Median values for densities (ind/m²) (range) of *Diadema antillarum* for all five transects at each depth interval surveyed in the five reef sites near La Parguera, Puerto Rico during 2001. Different small letters (a,b) on top of bars show significant differences in size distribution between populations of *D. antillarum* at the different depth zones (Dunn's, P<0.05).

shallow waters was higher than 1. Ratios higher than 1 were found for the intermediate waters and deep waters. All of the ratios were higher than 1, which means that populations of *D. antillarum* in La Parguera tend to have an aggregated pattern in all depth intervals sampled. The same aggregated distribution pattern was observed for each individual reef.

A Pearson Correlation indicated a positive, but not significant relationship between densities of *D. antillarum* and the complexity of the reef substrate or rugosity for all reef sites (Fig 4). The test revealed that at these density levels, rugosity did not have a significant effect on urchin populations ($P > 0.05$) on any of the sites but a trend to higher densities in more complex areas was observed (Fig 5).

III-2. Mean size and population size structure of *Diadema antillarum* in La Parguera.

The average size ($\pm$ SD) for *D. antillarum* around the entire study area of La Parguera, Puerto Rico in 2002 was 6.71 cm ($\pm$ 0.25) (Fig 6). Urchins with a maximum test diameter between 5 and 9 cm were most abundant and very few urchins larger than 9.1 cm or smaller than 4.9 cm were found (Table 5). Small individuals were observed in all of the reefs, indicating that a new generation of juveniles successfully recruited recently (Fig 7). No urchins larger than 10.5 cm were found during this survey.

Mean size (SD) of urchins was not significantly different across the same depth intervals with an average size of 6.95 cm ($\pm$ 0.13) in shallow fore reef areas, 6.48 cm ($\pm$ 0.65) at intermediate depths, and 3.37 cm ($\pm$ 2.9) in deep waters. The urchin size

frequency distribution in shallow waters was significantly different (K-S, P<0.05) than

the size distribution at intermediate depths where high numbers of smaller and less larger
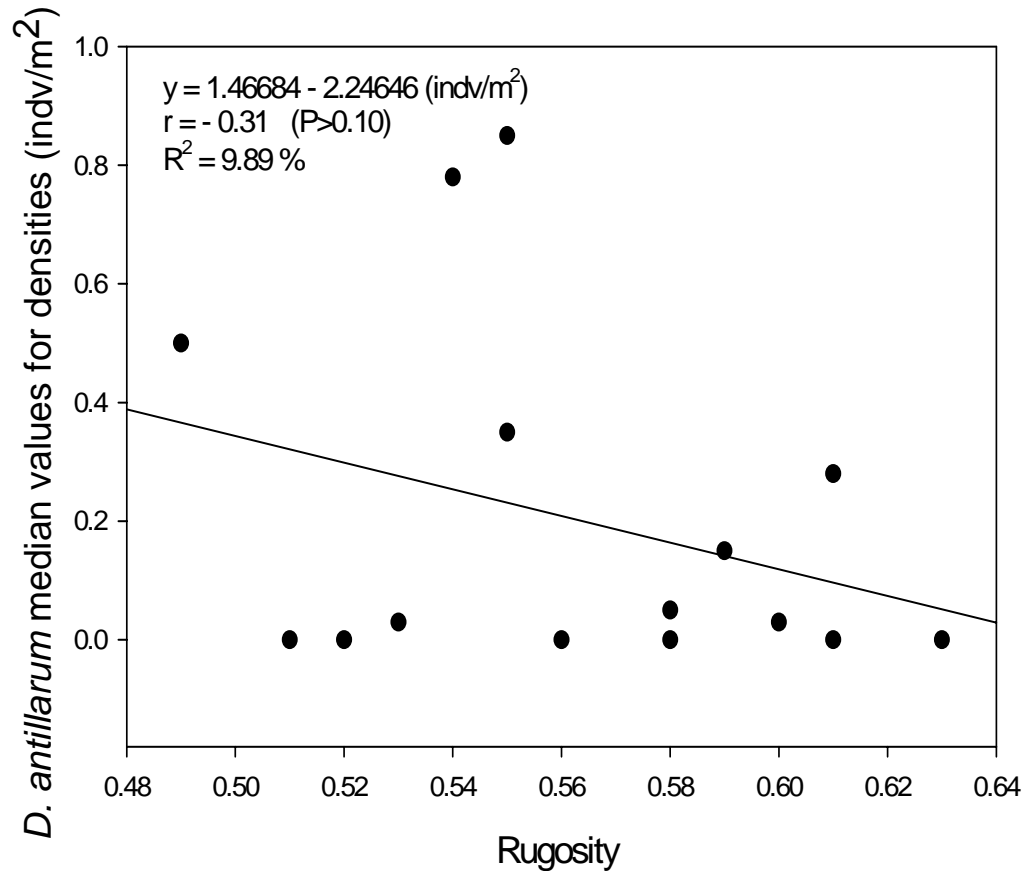
Figure 4. Linear regression between the median densities (ind/m²) of *Diadema antillarum* and rugosity for the entire area surveyed near La Parguera, Puerto Rico during 2001. No significant relationship between rugosity and densities of *D. antillarum* was found.

urchins were observed. The size distribution of *D. antillarum* in deep waters was not

significantly different (K-S, P≥ 0.05) when compared to both the distributions in shallow

and intermediate depths (Fig 8).
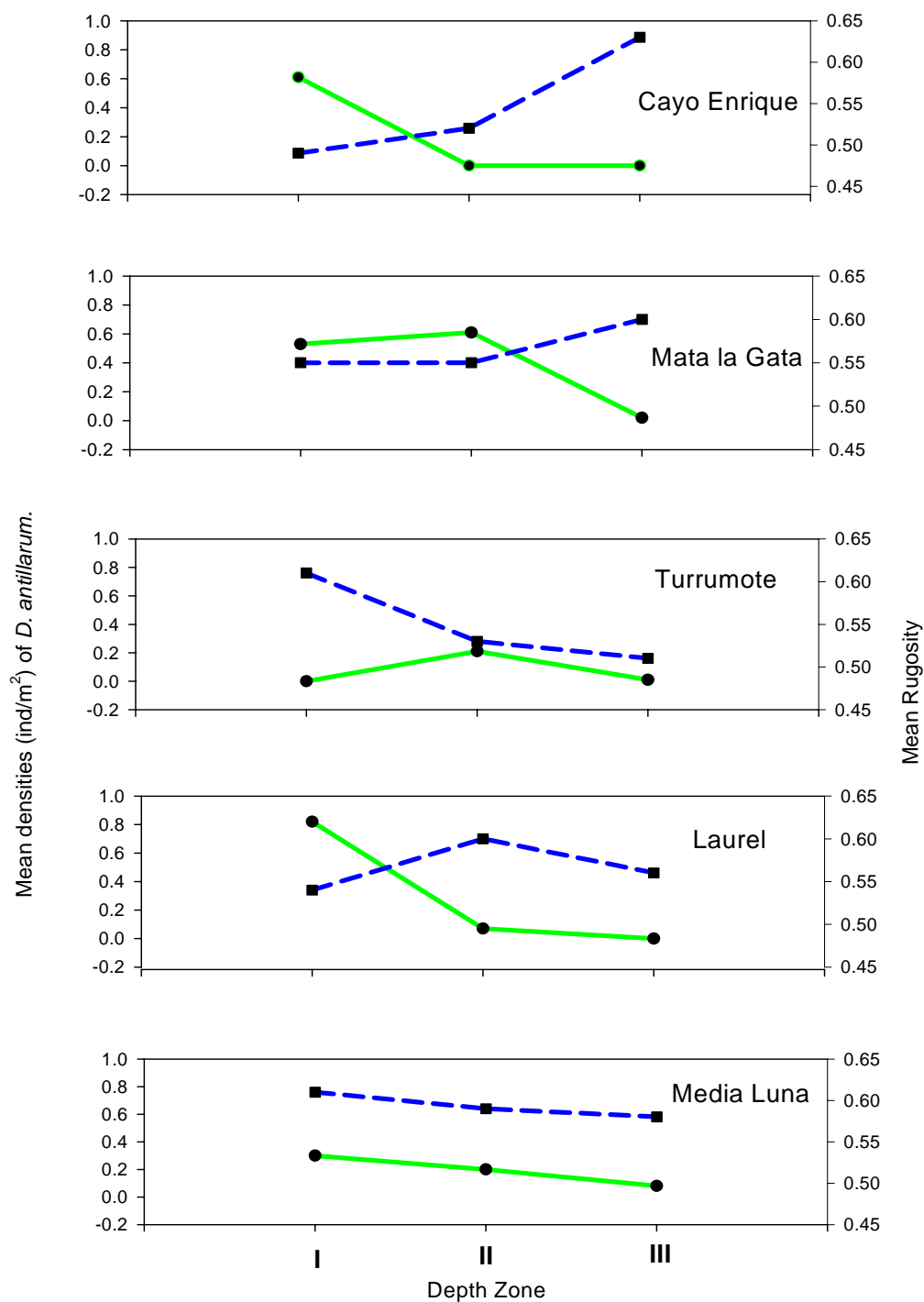
Figure 5. Relationship between mean densities ind/m² (●) of *Diadema antillarum* and mean rugosity (■) for each depth interval and each site sampled for La Parguera, Puerto Rico during the year 2001. Depth zone I = 0-3m, II = 4-10 m, III = > 10 m.
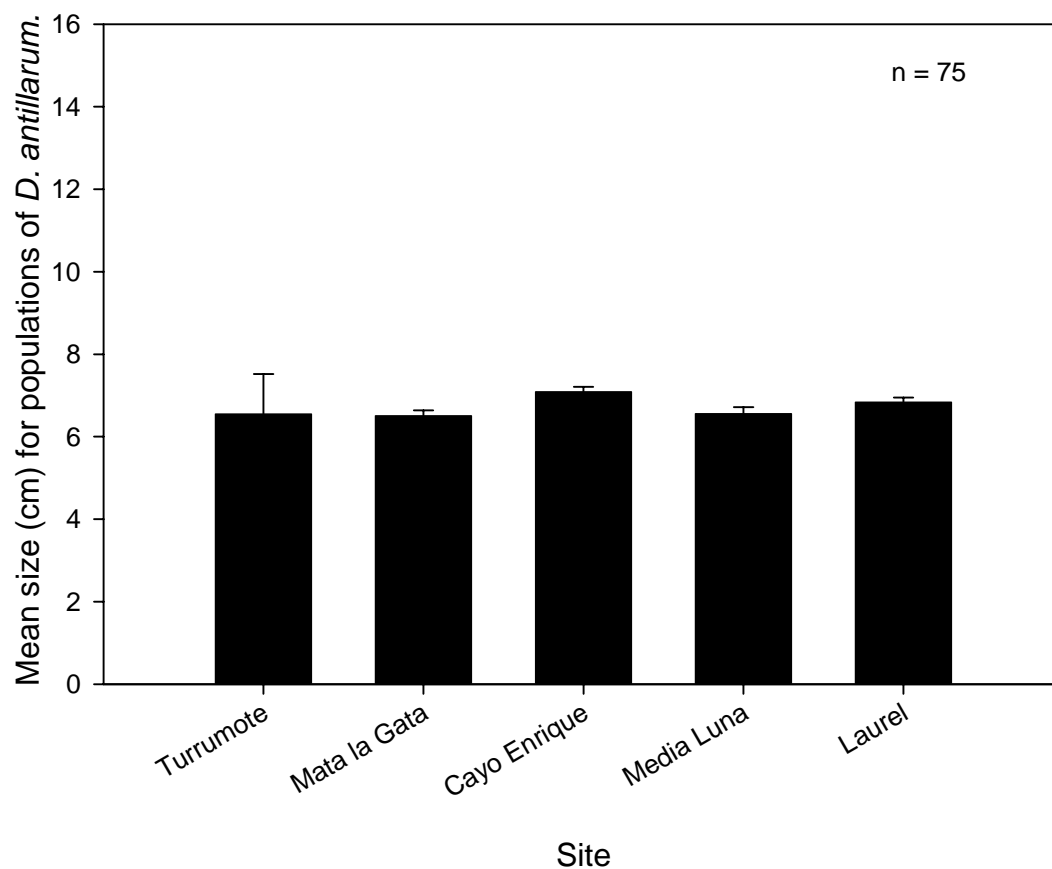
Figure 6. Average mean size (cm) (± standard error, n = 75) of *Diadema antillarum* for each of the reef sites surveyed in La Parguera, Puerto Rico for the year 2001. Significant differences in mean size (K-S, $P < 0.05$) across sites were only found between Media Luna and Cayo Enrique.
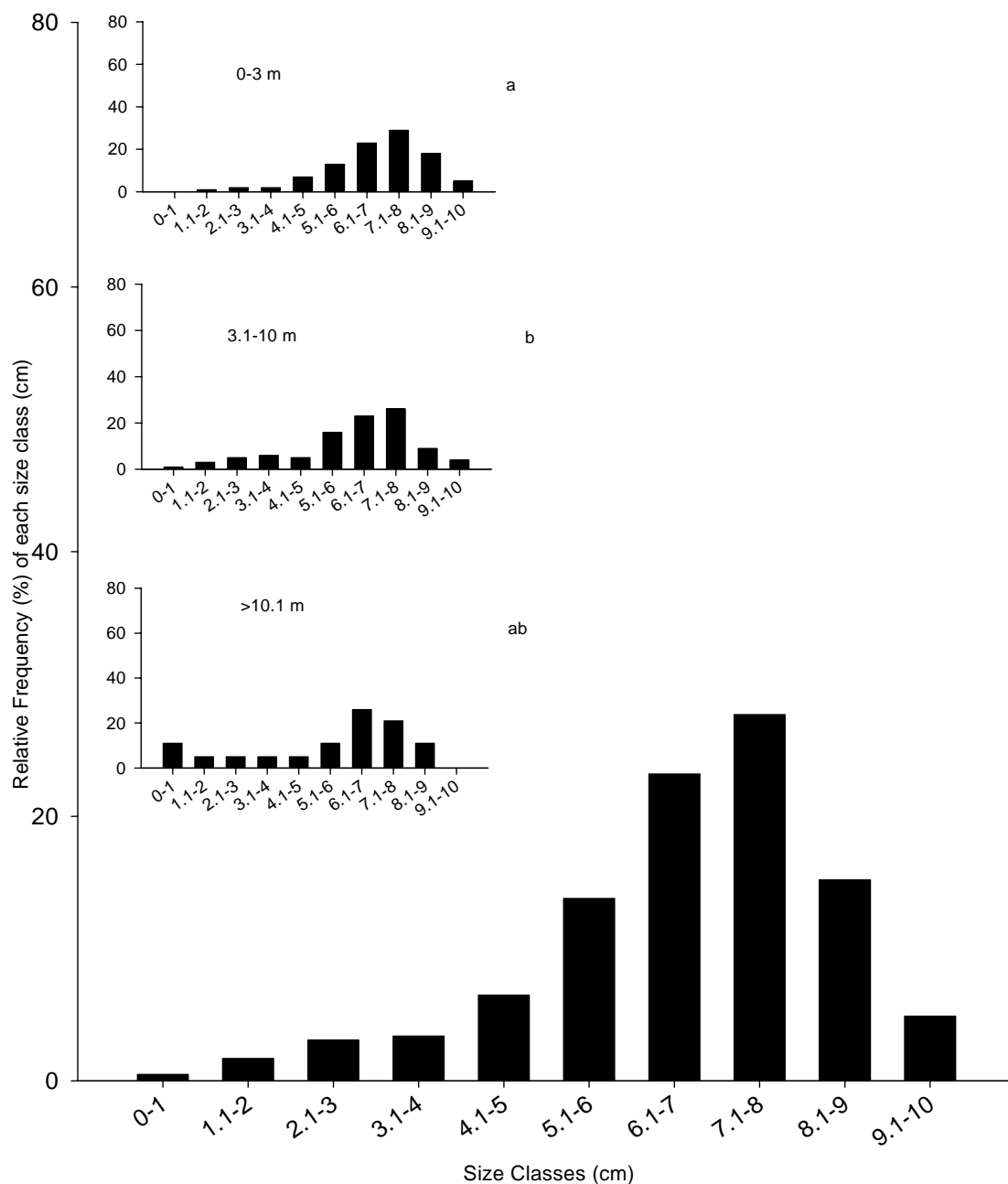
Figure 7. Population size frequency for *Diadema antillarum* pooled for all the sites and depths surveyed near La Parguera in 2001. Small figures represent the size frequency distribution of subpopulations found at each depth interval. A Kolmorogov-Smirnoff test showed that the urchin size distribution is significantly different (P<0.05) between the depth zones (shown by small letters: a,b).
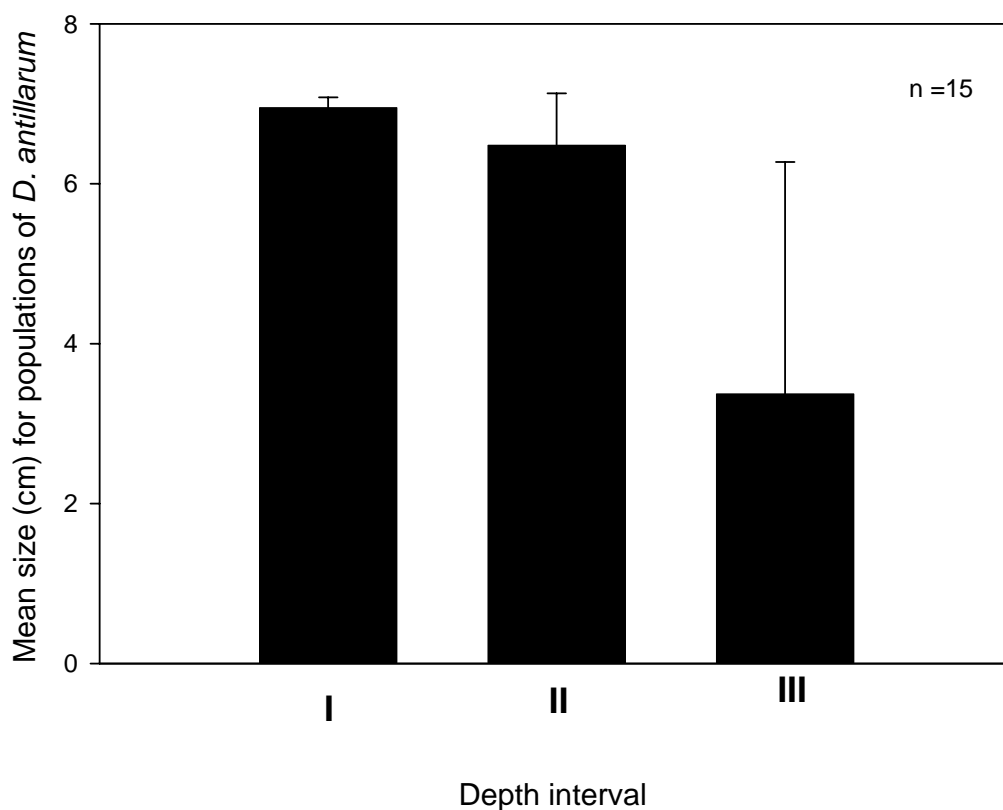
Figure 8.   Mean size (cm) (± standard error, n = 75) of *Diadema antillarum* for all five reefs at each depth interval for all the urchins surveyed in La Parguera, Puerto Rico in 2001.

The size frequency distribution for Cayo Enrique reef showed a high number of medium-large urchins compared to small, medium and very large individuals (Fig 9). At Mata la Gata reef, medium to large size individuals dominated the shallow and intermediate depth zones except on the deep zone where only small individuals were observed (Fig 10). Medium to large size urchins were also the most abundant in Laurel reef (Fig 11). Media Luna showed the same size frequency distribution between the three

depth intervals being dominated by medium to large individuals (Fig 12). At Turrumote reef, most urchins observed were in the medium to large range (Fig 13).

Of all the reefs surveyed, Mata la Gata and Laurel were the only reefs with a significantly different size distribution (K-S, P<0.05) between depth intervals where larger urchins decrease in number as we moved into deeper waters. The test was not run for Turrumote and Cayo Enrique due to the absence of *D. antillarum* in some depth intervals. No significant difference (K-S, P≥0.05) in mean size of *D. antillarum* between depth zones was recorded for Media Luna. The different mean size found between the different depth zones leads us to reject the H_{02}= *Depth and site influence the size distribution of D. antillarum*).

No significant difference (K-S, P≥ 0.05) in the size distribution of *D. antillarum* was observed between the five sites in shallow waters. Turrumote reef was omitted from this test due to the fact that no urchins were present in shallow waters. A comparison of *D. antillarum* size distribution in the intermediate depth zone revealed that there was no significant difference (K-S, P≥ 0.05) in mean size at this depth between reef sites. Cayo Enrique reef was omitted because no urchins were found at this depth interval. Only Media Luna and Mata la Gata were tested for a difference in size distribution at deeper depths because the other reefs did not have urchins at this depth. Results revealed a significant difference in mean size of *D. antillarum* between Media Luna and Mata la Gata (K-S, P<0.05). The urchins seen in the deeper waters of Media Luna were larger in size than the ones found in Mata la Gata, which had many small size individuals at this depth.
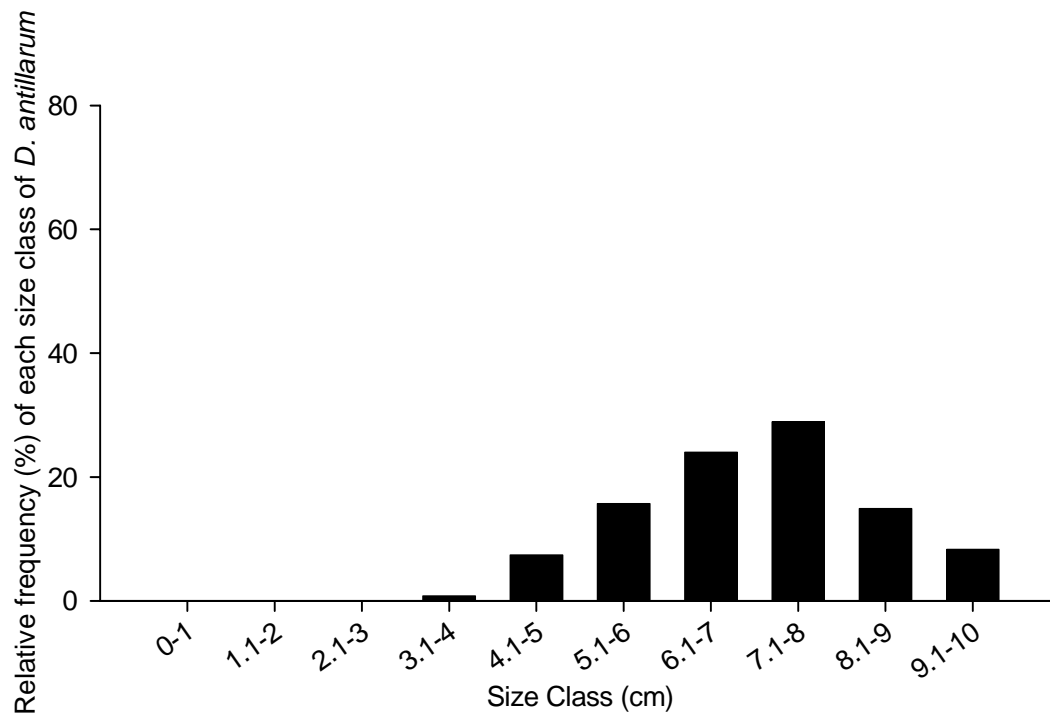
Figure 9. Population size frequency of *Diadema antillarum* at the Cayo Enrique reef site during the year 2001.

A Pearson Correlation performed between mean size of *D. antillarum* and the complexity of the reef floor, or rugosity, in each reef site revealed that rugosity did not have a significant effect on the urchin size distribution ($P > 0.05$) at any of the sites.

A linear regression showed that there is a significant relationship between the mean size and mean densities of *D. antillarum* ($P < 0.05$) (Fig 14, Plot A). But when a second linear regression was performed without the outliers, no relation was found between urchin densities and size (Fig 14, Plot B). This suggests that the size of the urchin is not influenced by urchin densities but rather by depth, where small urchins recruit in deep waters and then move to shallow water after reaching a certain size.
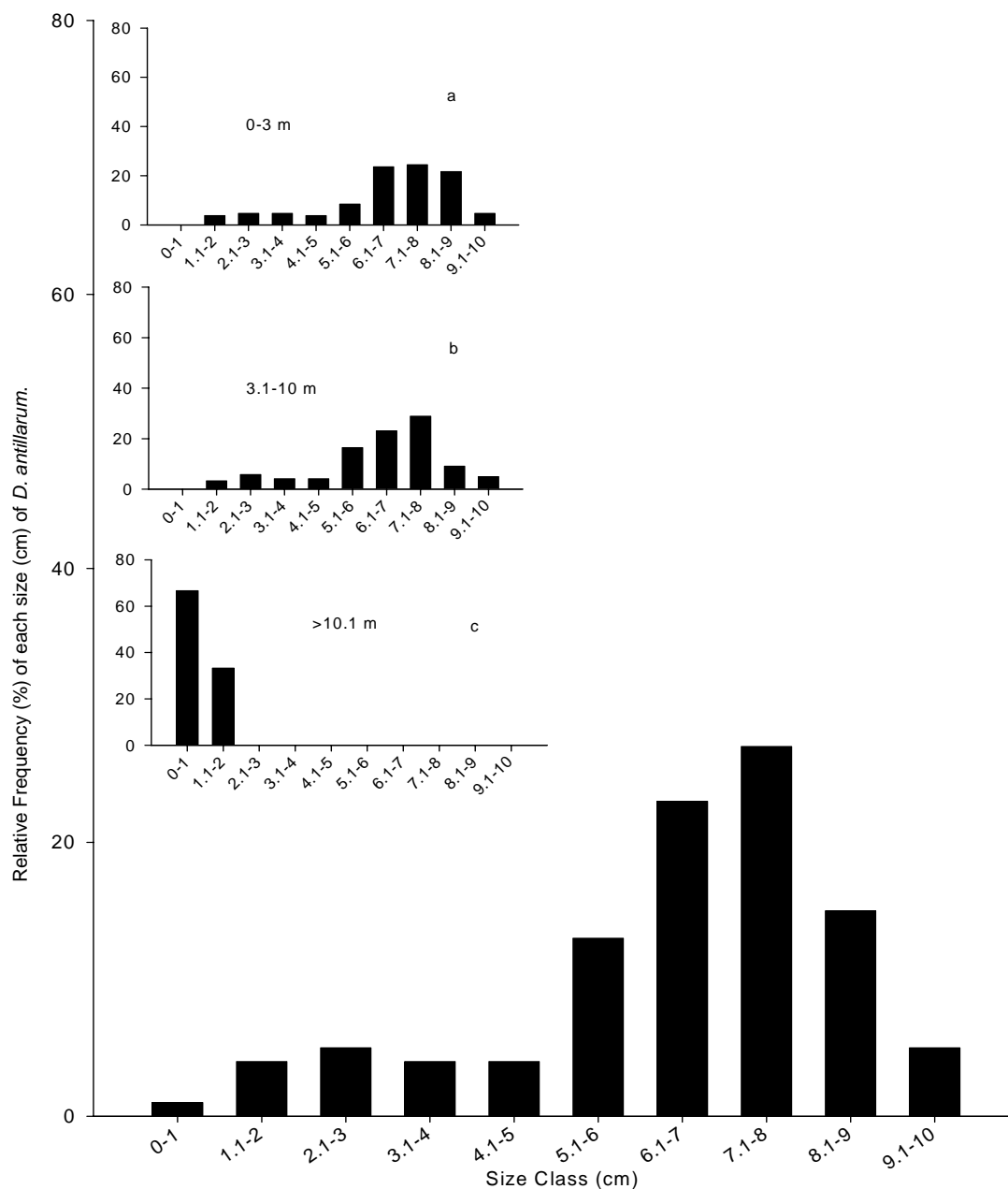
Figure 10. Population size frequency for *Diadema antillarum* at Mata la Gata in 2001. Small figures to the left show the urchin size frequency distribution by depth interval. The size distribution for the entire area is expressed in the large graph. Urchin size distribution was found to be significantly different (K-S, P<0.05) between the depth zones (shown by small letters: a,b,c).
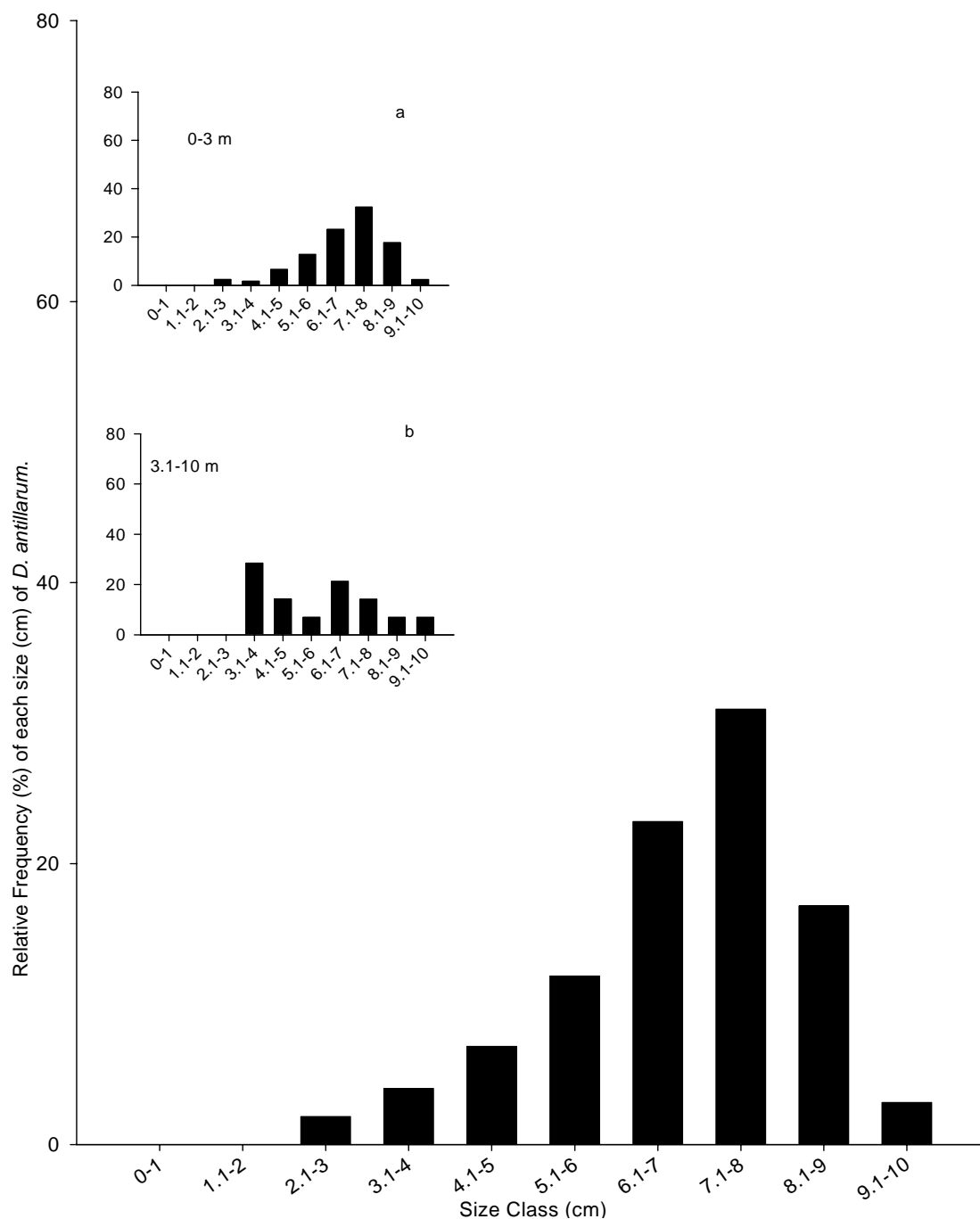
Figure 11. Population size frequency for *Diadema antillarum* at Laurel in 2001. Small figures to the left show the urchin size frequency distribution by depth interval. The size distribution for the entire area is expressed in the large graph. Urchin size distribution was found to be significantly different (K-S, P<0.05) between the depth zones (shown by small letters: a,b,c).
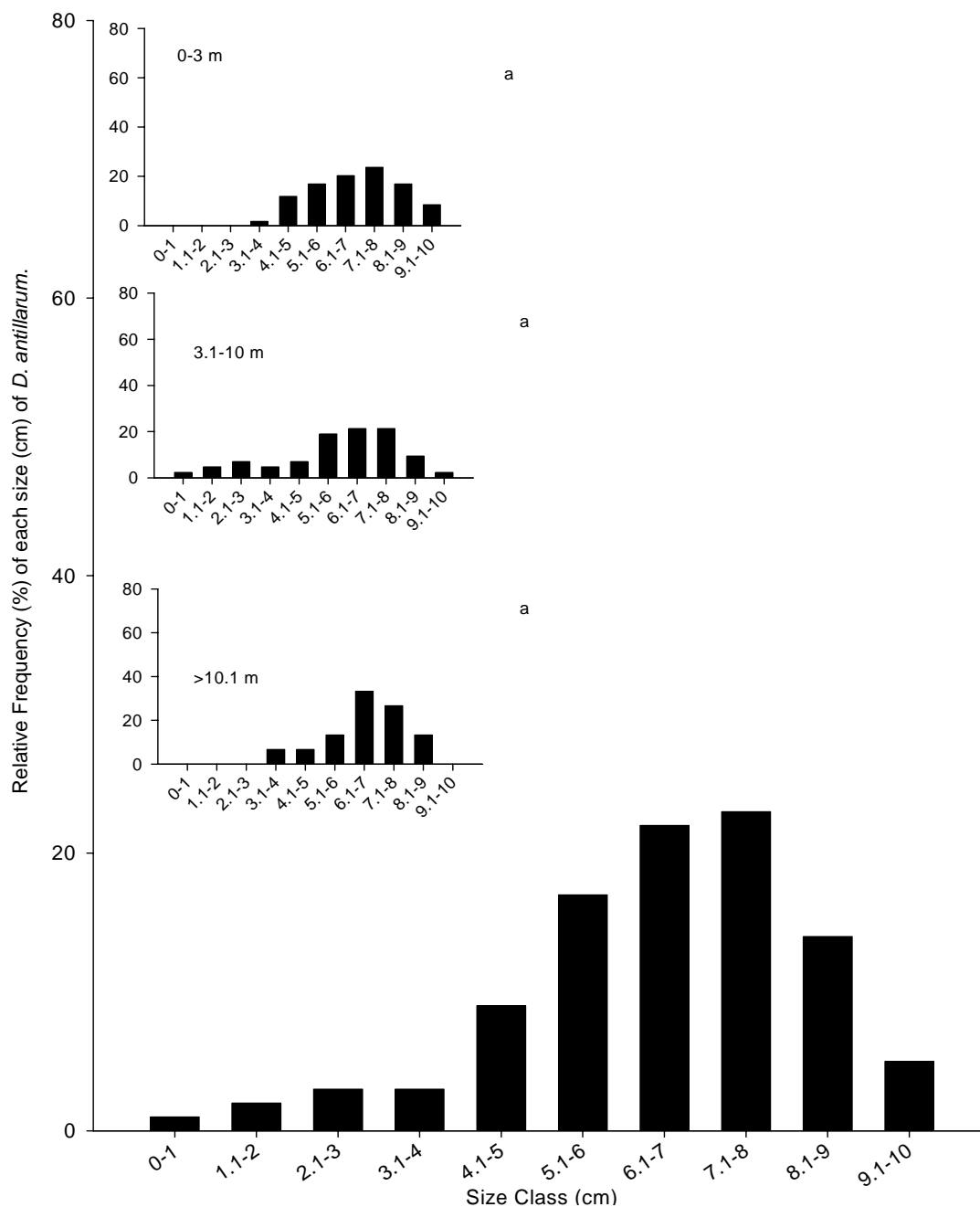
Figure 12. Population size frequency for *Diadema antillarum* at Media Luna in 2001. Small figures to the left show the urchin size frequency distribution by depth interval. The size distribution for the entire area is expressed in the large graph. Urchin size distribution was not significantly different (K-S, P>0.05) between the depth zones.
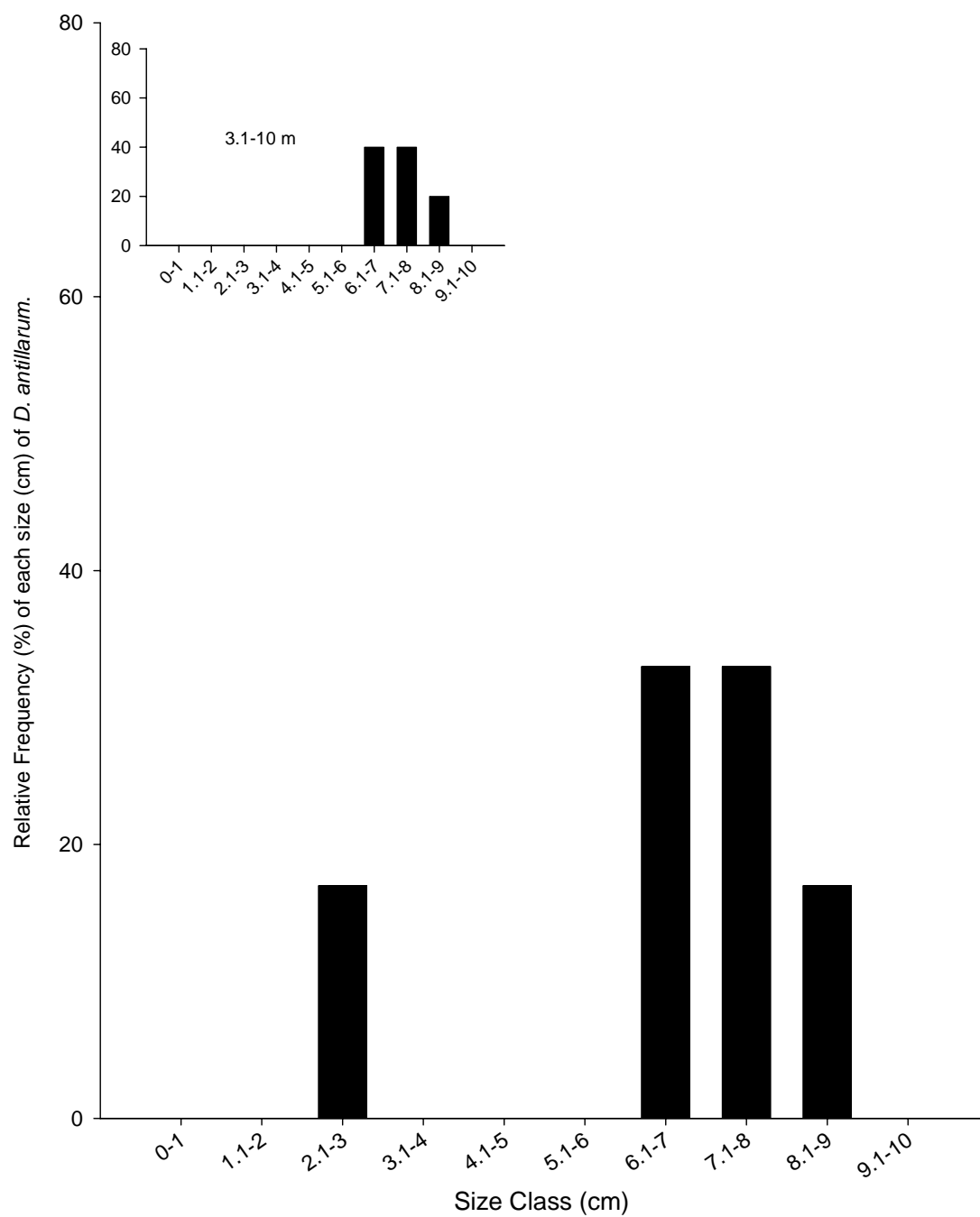
Figure 13. Size frequency for *Diadema antillarum* at Turrumote in 2001. Small figures to the left show the urchin size frequency distribution by depth interval. The size distribution for the entire area is express by the large graph.
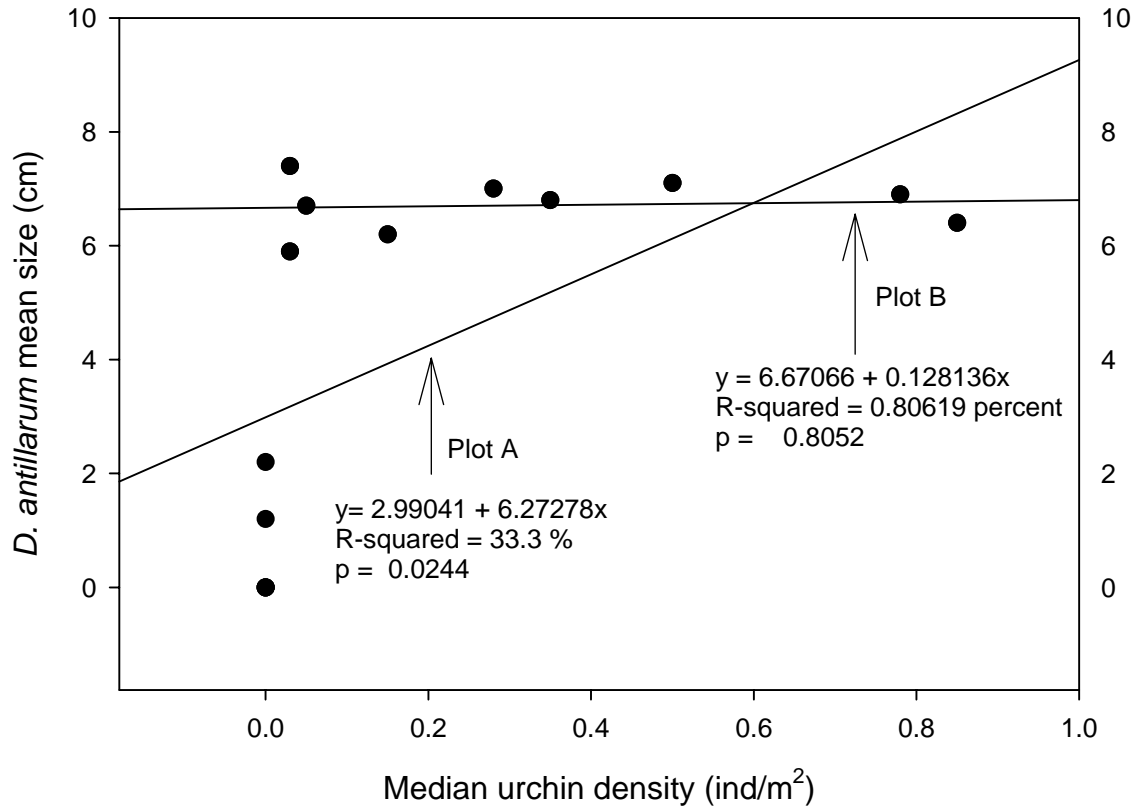
Figure 14. Linear regression between mean size (cm) of *Diadema antillarum* and median densities for the entire area surveyed near La Parguera, Puerto Rico during 2001. A significant relationship between median densities and mean size of *D. antillarum* was found (P= 0.0007) in Plot A but not in Plot B were the regression was performed without the outliers.

III-3. Reproductive Traits of *Diadema antillarum* in Puerto Rico.

During the study period, the mean gonad index (GI) ranged from a low 2.9 (June-July 2001) to a high of 11.2 (December 2001) (Fig 15). High gonad indices were registered for the late fall (9.5 GI) and the winter (8.9 GI) when the sea surface temperature (SST) was at its lowest value (26°C). A significant decrease (One Way

ANOVA, P<0.05) in gonad index (4.2 GI) was recorded during the early spring and summer (Table 6). A slow increase in the gonad index started again in the late fall. Moore et al. (1963) suggested that an abrupt decrease in the gonad index is a sign of the spawning period of the urchins, which was the case for spring and early summer in La Parguera. The increase in the gonad index seen during the fall indicated a new cycle peak in reproductive activity for the urchin populations in La Parguera, which peaked in the late winter and early spring, when the massive spawning occurred. Urchins used stored energy for gametogenesis, and then released their gametes during the spring.

Weekly data for sea surface temperature (SST), salinity and visibility was obtained from the CARICOMP stations in La Parguera and correlated to the mean weekly gonad index from November 2001 to November 2002 to determine if there was a significant relationship between reproductive cycle of *D. antillarum* and SST. A Pearson Correlation showed that there is a significant, but negative, relationship (r= -0.71) between monthly mean gonad indexes and SST (Fig.16). This means that urchins have larger gonads during lower water temperature periods and smaller ones at high SST for La Parguera. The SST during the period with the highest gonad index was around 27.5 °C and was 28.7 °C during the lowest mean gonad index. Average weekly salinity and turbidity were obtained for each of the weeks when urchin gonad index was determined (Table 7). No significant relationship (P>0.05, Pearson Correlation) was found between GI and salinity or GI and turbidity over the study period.
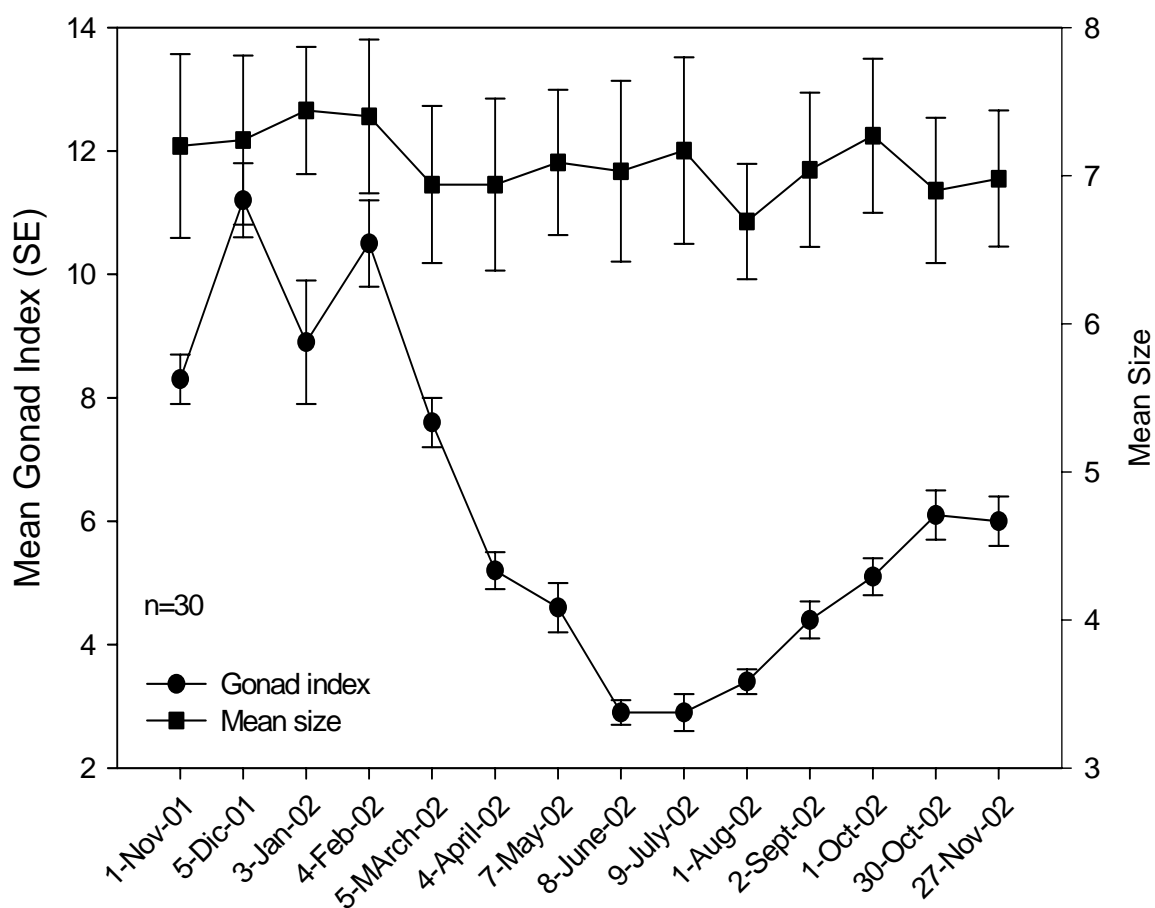
Figure 15. Monthly mean gonad index vs. mean size (cm) for thirty *Diadema antillarum*. Urchins between 6.1-8 cm size classes were chosen at random from November 2001 to November 2002 in La Parguera, Puerto Rico.

Table 6. Temporal variability in mean gonad index and weight (g) of *Diadema antillarum* for size class interval 6.1-8 cm during the months of November 2001 to November 2002 near La Parguera, Puerto Rico.

Month	Gonad Index (SD)	Mean Weight (SD)	Mean Size (SD)
1-Nov-01	8.3 (1.9)	140.7 (33.7)	7.2 (0.62)
5-Dec-01	11.2 (2.9)	161.6 (33.6)	7.24 (0.57)
3-Jan-02	8.9 (3.9)	162.2 (30.3)	7.44 (0.43)
4-Feb-02	10.5 (3.3)	176.8 (32.5)	7.4 (0.52)
5-Mar-02	7.6 (2.3)	162.7 (40.6)	6.94 (0.53)
4-Apr-02	5.2 (1.3)	164.4 (38.7)	6.94 (0.58)
7-May-02	4.6 (2.0)	173.5 (36.2)	7.09 (0.49)
8-Jun-02	2.9 (1.3)	157.3 (38.4)	7.03 (0.61)
9-Jul-02	2.9 (1.2)	168.2 (40.8)	7.17 (0.63)
1-Aug-02	3.4 (0.9)	143.9 (27.5)	6.69 (0.39)
2-Sep-02	4.4 (1.5)	168.9 (37.5)	7.04 (0.52)
1-Oct-02	5.1 (1.6)	186.0 (37.5)	7.27 (0.52)
30-Oct-02	6.1 (2.0)	176.0 (35.0)	6.9 (0.49)
27-Nov-02	6.0 (1.9)	187.0 (31.1)	6.98 (0.46)

Urchins of all sizes were dissected to determine the smallest reproductive size. The smallest sexually mature urchin in La Parguera was 3.8 cm in diameter. Twenty other individuals, close to 3 cm in size, were dissected and none had gonads. We found that on some occasions, urchins between 4 and 5 cm also lacked gonads.

After performing the histological analysis of the gonads female (Fig 17) and male (Fig 18) reproductive cells were distinguish. The male:female ratio of *D. antillarum* in La Parguera was 0.89:1 during our observations (2001-2002) (Table 8).
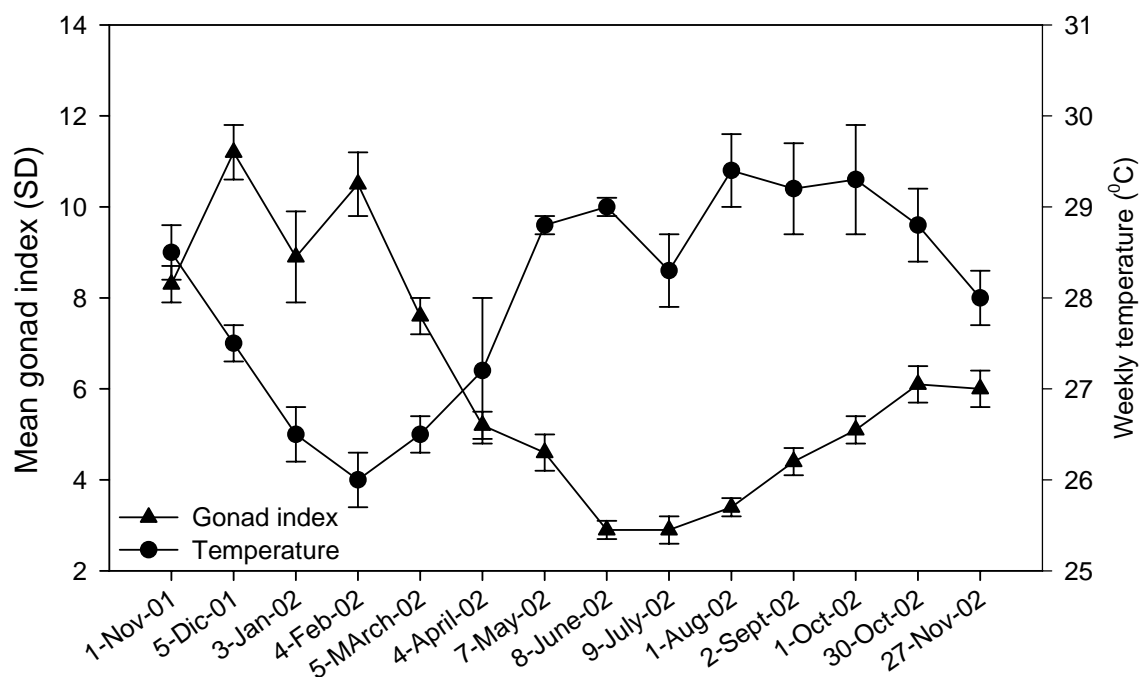
Figure 16. Mean gonad index (●) of *Diadema antillarum* vs. mean weekly temperature (C°) (■) between November 2001 and November 2002 of La Parguera, PR. (P<0.05, linear regression)
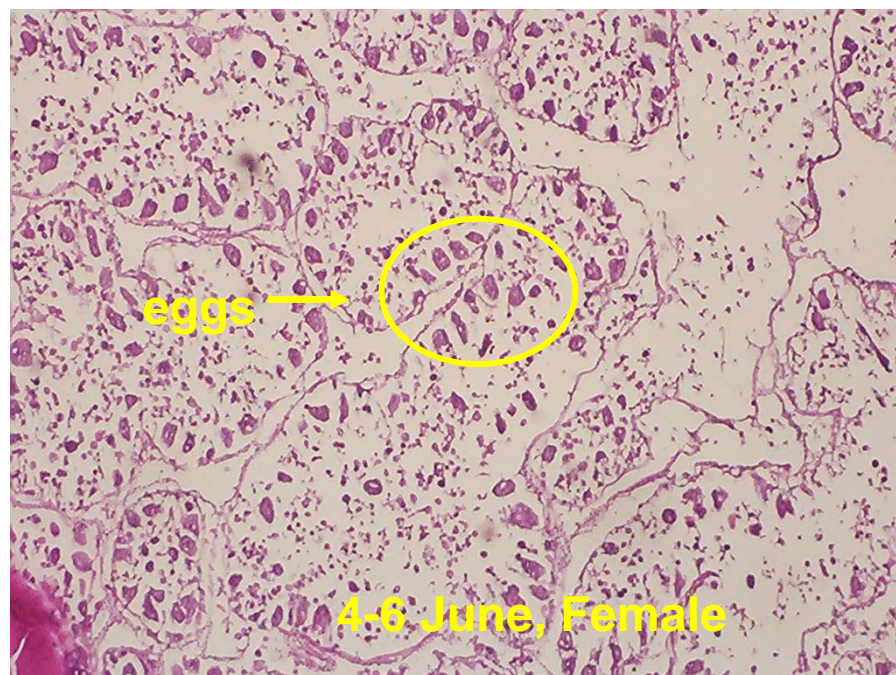
Figure17. Photos of female *Diadema antillarum* reproductive structures (gonads). The circles indicate the eggs inside the gonads.
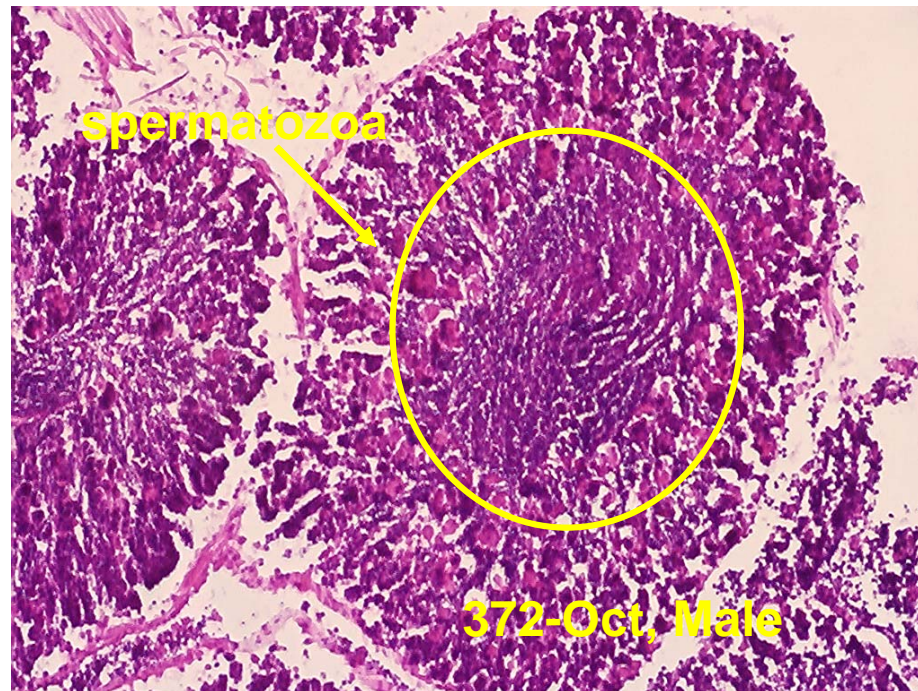
Figure18. Photos of male *Diadema antillarum* reproductive structures (gonads). The circles indicate the sperms inside the gonads.

Table 7. Temporal variability in mean gonad index of *Diadema antillarum* populations, temperature (°C), salinity (PSU) and turbidity (horizontal/Secchi) between November 2001 and November 2002 near La Parguera, Puerto Rico.

Month	Mean G.I	Average Weekly Temp (°C)	Salinity (PSU)	Turbidity
1-Nov-01	8.3	28.5 (0.4)	36 (0.6)	8 (0.0)
5-Dec-01	11.2	27.5 (0.2)	36.5 (0.2)	12 (2.5)
3-Jan-02	8.9	26.5 (0.3)	38 (0.8)	12 (1.8)
4-Feb-02	10.5	26 (0.3)	38.5 (0.3)	15 (2.7)
5-Mar-02	7.6	26.5 (0.2)	38.5 (0.3)	15 (2.4)
4-Apr-02	5.2	27.2 0.8)	39 (0.9)	15 (2.9)
7-May-02	4.6	28.8 (0.1)	38.5 (0.3)	10 (3.0)
8-Jun-02	2.9	29 (0.1)	37.8 (0.5)	15 (4.2)
9-Jul-02	2.9	28.3 (0.4)	36 (0.5)	5 (2.1)
1-Aug-02	3.4	29.4 (0.4)	37 (1.0)	3 (3.7)
2-Sep-02	4.4	29.2 (0.5)	35 (0.0)	15.3 (3.4)
1-Oct-02	5.1	29.3 (0.6)	35 (0.2)	7.5 1.8)
30-Oct-02	6.1	28.8 (0.4)	36 (0.0)	6 (2.5)
27-Nov-02	6	28 (0.3)	36 (0.3)	9.8 (1.7)

Table 8. Sex ratios (♀/♂) for populations of *Diadema antillarum* surveyed each month from June to November of 2002 near La Parguera, Puerto Rico.

Month	Male	Female	Ratio
June	6	4	
August	1	0	
9-Sept-02	4	6	
16-Sept-02	2	8	Male:Female
23-Sept-02	3	7	
1-Oct-02	7	6	0.89:1
25-Oct-02	1	4	
30-Oct-02	3	2	
5-Nov-2	4	1	
12-Nov-02	2	3	
25-Nov-02	3	2	

Table 9. Comparison of size frequency distribution between the three depth zones within each reef. Significant difference (P<0.050) was found in depth intervals of Turumote, Mata la Gata and Laurel. The (yes) = indicates that significant differences were found, (no) indicates that no significant difference was found between the two depth intervals and (----) = no urchins = data was available to make the comparison. I = 0-3 m, II = 4-10 m, III = > 10 m.

Site	Depth I x II	Depth II x III	Depth I x III
Turrumote	no	yes	yes
Mata la Gata	no	yes	yes
Cayo Enrique	-----	-----	-----
Media Luna	no	no	no
Laurel	yes	-----	-----

IV. Discussion

Recent densities reported for the Parguera area by Torres et al. 2000 and the ones observed during this survey, indicate that after nineteen years of the *Diadema antillarum* die off, urchin populations around La Parguera, Puerto Rico are showing a slow recovery. Population densities still remain significantly lower than pre-mortality densities. Urchin densities in La Parguera before 1982 were similar to those reported in other Caribbean localities (5-12 ind/m², Craft 1975). Vicente and Rivera (1984) reported *D. antillarum* densities of 0.13 ind/m² after 1983 in Jobos Bay, Puerto Rico. Other localities around the Caribbean such as Jamaica and Florida, which reported high urchin densities before the die-off, have also experienced a slow recovery of *D. antillarum* since 1983. Current densities in those locations are similar (ranged from 0 to 0.6 ind/m²) to the ones found in this study (0 to 0.83 ind/m²) (Cho et al. 2001, Edmunds et al. 2001, Haley et al. 2001, Moses et al. 2001, Chiappone et al. 2002). Unpublished records by Lynda Craft (1975) indicated that *D. antillarum* had a depth distribution ranging from 0.25 to 10 m depth in Turrumote and as deep as 25 m at Media Luna. In 2001, *D. antillarum* in Turrumote and Media Luna reef can still be observed at the same depths reported previously, but with very low densities.

Current *D. antillarum* populations in La Parguera mostly inhabited shallow reef habitats (0-3 m), except at Turrumote reef where the structure of the reef and the degree of exposure seem to favor the distribution of urchins from intermediate to deep habitats. High variability in population densities and distribution was evident by the significant differences in densities found across the reef localities for the same depth intervals. The lack of a significant correlation between the substrate rugosity and the mean urchin

densities could mean that urchins had no preference for more complex habitats that will provide shelter and protection from predators. Urchin densities in 2001 were still very low and they had not yet populated most of the high complexity areas. Results were no significant but observations in this study indicate a general trend of highest densities and smaller urchins inhabiting more complex habitats. Previous data for other reef localities in La Parguera supports this result (Weil et al. in press).

In shallow and exposed (wave action) zones around the Caribbean, low densities of urchins were recorded compared to more protected areas (Randall 1964). Exposure to wave action could be the reason why Turrumote had the lowest density counts of urchins in shallow habitats in 2001. Craft (1975) reported that Turrumote had a high cover of elkhorn coral (*Acropora palmata*) and also presence of high numbers of *D. antillarum* before 1983 in shallow habitats. Populations of *A. palmata have* decreased dramatically in Turrumote after events like hurricane David and the white band disease epizootic event of the early 1980's (Gladfelter 1984). When the Acroporid populations died, bioerosion and wave action crumbled the dead Acroporids crumbled, significantly reducing the the spatial heterogeneity and therefore, the availability of hiding places and protection for small urchins. Haley et al. (2001) suggested that the fore reef habitat in many Caribbean reefs is less complex now than in the early 1980's, because of the loss of *A. palmata* and *A. cervicornis*, which reduced shelter for *D. antillarum* recruits and juveniles against predators.

Mortensen (1937), Hawkins (1982), Leis (1982), Weil et al. (1984), Goldman (1984), Hunte & Yunglao (1998) and others have suggested various reasons to explain the significant difference of urchin densities around the tropical Caribbean reefs. Reefs

that had high densities of *D. antillarum* before 1983 could recover faster than those with lower initial densities assuming a proportional rate of mortality. Hunte and Yunglao (1998) proposed various alternatives to this hypothesis, the first one being that recruits in a given area are produced by a spawning event on the same area. Hence, more breeding adults on the area would produce more larval recruits to that reef over time. The problem with the proposed hypothesis is that the reefs surveyed in La Parguera were too close together, and *D. antillarum* has a lengthy planktonic larval stage (4 to 6 weeks). Under these conditions, this hypothesis could apply only if local recruitment is promoted by local eddies keeping the larvae within La Parguera reef system. The second hypothesis of onshore currents returning larvae to reefs might apply more directly to certain reefs than others (Mortensen 1937, Hawkins et al. 1982). Hence, some reefs will have a higher recruitment rate than others, which seems to be the case in La Parguera. A third possibility is that reefs vary in quality in terms of availability of resources per unit area, and this enhances recovery through lower post-settlement mortality. Finally, if planktonic larvae actively choose reefs with populations of *Diadema* on them, then larvae of *D. antillarum* might settle on reefs with higher adult densities (Leis 1982, Leis & Goldman 1984).

Assuming that recruitment was uniform, high densities of *D. antillarum* observed on shallow waters and not in deeper waters off La Parguera could be the result of high post-recruitment survival due to structural complexity and lack of predators resulting in higher densities of urchins (Ebert 1982, Bak 1985). In La Parguera, urchin densities showed a significant reduction with increasing water depth, with few or no urchins in waters deeper than 10 m on most reefs. A reduction in densities with decreasing reef

complexity was also observed. A similar lack of urchin in deep waters has been reported for Florida and Jamaica (Cho et al. 2001, Edmunds et al. 2001, Haley et al. 2001, Moses et al. 2001, Chiappone et al. 2002). This could be related to the lack of algae biomass and diversity that will affect the feeding patterns of this urchin. The deep fore reef zones at Laurel, Cayo Enrique and Media Luna, were dominated by muddy areas with consolidated substrate, scattered coral colonies, and, as expected, a low abundance of urchins.

Densities of *D. antillarum* in areas dominated by *Millepora spp.*, *Montastraea spp.* and *Acropora spp.* were higher than in other reef habitats. This was the case at Cayo Enrique, Laurel and Mata la Gata where deep fore reef areas were dominated by mud (silt and clay) and macro algae. Meanwhile, in the middle depth zone (3-10 m) were the other massive (*Montastraea spp., Siderastrea spp.*) plate (*Meandrina spp., Mycetophyllia spp.*) and the branching (*Porites spp.*) species were present, higher densities of urchin were observed but lower than those observed in the shallow zone. The shallow zones provided more crevices for *D. antillarum* to hide than the intermediate and deep waters and the availability of food was much higher. A very unlikely place for the presence of *D. antillarum* was the area dominated by soft corals, as was the case of the shallow platform of Turrumote, where a high dominance by gorgonians and no *D. antillarum* were found. The area covered by gorgonians may be less attractive to the urchin because of the lack of protection due to low abundance of crevices.

After 1983, reef sites with high numbers of urchins had a higher recruitment and recovery rate than those that had low numbers originally (Hunte et al. 1988). Migration to deep waters by *D. antillarum* in 2001 might have not happened because of low levels of

competition for space and food in the shallow fore reef waters (Randall 1964). In addition, the low numbers of urchins at deep zones could result from low recruitment at these depths (Bak 1985). Even if there is higher recruitment at deep areas, urchins would probably move to the more illuminated habitats where food is more readily available.

Assuming that reproduction success is inverse density-dependent, only when adult populations are extremely low will the number of larvae surviving the plankton stage be inadequate to allow a local population to increase (Hunte et al. 1988). Recovery of *D. antillarum* populations for La Parguera, could be accelerated when the parental stock size recovers to the point where it increases the opportunity for external fertilization (Hughes 1994, Lessios 1988). At La Parguera, no prediction could be made as to when the populations would reach the right parental stock needed for a fast recovery of the urchin populations. There is the possibility that the low water quality in some areas (caused by non-point sources of pollution), may be affecting the chances of survival of the urchin larvae as it has been reported for coral larvae (Richmond, personal communication). Poor recruitment surfaces due to increased algal cover, post settlement mortality or inter-specific competition (Bak 1985) are also important factors that could affect recovery of the urchin populations in La Parguera.

Most of the populations of *D. antillarum* surveyed showed an aggregated distribution pattern over the reef. *D.* antillarum aggregations in open areas seem to be a defense mechanism against predation and have been reported several times in other populations around the Caribbean. These aggregations also occur as a mechanism for protection from UV rays and during periods of spawning (Randall, 1986).

In La Parguera, populations of *D. antillarum* showed a size distribution with predominant medium to large sizes (6-8 cm) and very few small (< 5 cm) or very large individuals (>10 cm). Previous data for Puerto Rico showed a similar size frequency distribution with predominance of individuals in the 6-8 cm size range (Vicente et al. 1984, Torres et al. 2001).

This size distribution may indicate a low level of recruitment or juvenile survival, a high mortality of either juveniles or small adults or a rapid growth of *D. antillarum* (Randall 1964). Hunte et al. 1988 and Carpenter 1990 provide other explanations for the lack of small and large urchins: 1) low numbers of large urchins could be the result of normal processes of senescence and mortality; and 2) low abundance of urchins outside the cohort of 5-8 cm could be the result of low recruitment or high mortality that particular year. It is difficult to determine with certainty the reason for the urchin size distribution because of a lack of data for La Parguera from other years. The low densities of big sea urchins in deeper waters could be attributed to (a) the low complexity of the sea floor and, as a consequence, less crevices for hiding, (b) lack of intraspecific competition in shallower areas due to high abundance of algae and low urchin population densities. Some high numbers of small urchins in deep waters and low numbers in shallow waters may indicate that the urchins tend to recruit in deep waters and move up to shallower habitats as they reach a certain size.

Randall (1964) reported that there were at least fifteen fish, two gastropods and one crustacean that preyed on *D. antillarum.* Some of these species included *Balistes vetula* (triggerfish), *Trachinotus falcatus* (permit), *Diodon hystrix* (porcupine fish), *Bodioanus rufus* (wrasse), *Lactophrys bicaucadis* (trunk fish), *Haemulon plumier* (grunt),

Calamus calamus (porgies), *Panulirus argus* (lobster) and others. None of these species were actually abundant or were seen actively predating on *D. antillarum* during this study. The lack of juvenile urchins in the reef surveyed could be the result of differential larval pool and recruitment, differential predation or high mobility of the small urchins. Observations in other reefs and the back reef areas or lagoonal platforms around La Parguera indicate high densities of small urchins in shallow, complex habitats (Weil et al. in press).

The sex ratio for *Diadema antillarum* in La Parguera was close to the expected 1:1 ratio. However, there were fewer males than females in the sample analyzed. Similar sex ratios have been reported by Meidel et al. (1998) for *D. antillarum*, and for other urchin species *Strongylocentrotus droebachiencis* and *Evechinus chloroticus,* (Lamare et al. 2002).

Urchins smaller than 3.8 cm in diameter were considered juveniles since all of the urchins sampled that were smaller than 3.8 had no gonads and therefore, were not sexually mature. Similar results were reported by Bauer (1976) for urchins less than 3.5 cm. Populations described by Randall (1965) showed a variation in size at which maturity was reached. In the U.S. Virgin Islands, urchins between sizes 3.2 to 3.4 cm were already extruding eggs but, in populations in Florida, urchins between 3.0 and 6.7 cm were still immature (Randall 1965). Urchins in La Parguera showed similar variation in sexual maturity with size, with urchins as big as 5.0 cm being sexually immature. This shows that not all individuals of *D. antillarum* reach maturity at the same time during their life cycle assuming equal growth rates and sexual maturity can vary with size. The variation in reaching sexual maturity might be explained by the differential growth rates, where

urchins that grow faster are using up all their energy and resources to grow instead of using them to reproduce (Bauer 1976, Levitan 1989).

Spawning for *Diadema* species in tropical and subtropical regions varied with seasons and months (Randall 1964). Mortensen (1921) reported finding mature *D. antillarum* in late March around the island of Tobago. Harvey (1947) found mature urchins in summer and early winter. Similar time/season variation in spawning events of *D. antillarum* has been reported for the rest of the Caribbean by Randall et al. (1964), Lessios (1981) and others (Table 4). For 2001-2002, urchins near La Parguera followed the same spawning cycle than those populations in the U.S. Virgin Islands (Randall et al. 1964), Barbados (Lewis 1966) and Curacao (Iliffe et al. 1982). All of these localities are geographically within 4 degrees of latitude. In Florida and Bermuda, the spawning peaks maybe in a different season because they are both in different latitudinal ranges. Bermuda is located on the northern edge of the reef distribution (32º20' N, 64°45' West) with different seasonal environmental patterns. It seems that urchin populations in the USVI, Barbados and Curacao have synchronized spawning events with urchin populations in Puerto Rico. Urchins of the U.S. Virgin Islands may be supplying larvae, floating 4 to 6 weeks down current, to reefs in Puerto Rico.

Past observations in Puerto Rico indicated that most urchins were mature during the winter, a few were mature in the fall but, the peak of the spawning takes place during late spring. This is corroborated by the significant decrease in the gonad index (GI) in spring and summer. Results showed that there is a yearly reproductive cycle for *D. antillarum* populations in La Parguera. Gametogenesis started in late fall reaching a pre-spawning maximum when the sea surface temperature (SST) was the lowest. A

significant decrease in GI was recorded during May that indicates that most populations of *D. antillarum* spawned during the spring. In other populations of *D. antillaurm,* sudden decreases of GI have been interpreted as spawning (Moore et al. 1963b). Previous observations in Puerto Rico showed spawning of urchins in the month of February in 1963 (Randall 1964). Observations made by scientists in the island of Culebra, Puerto Rico showed a group of *D. antillarum* spawning during April 2003 (Ruiz, Personal communication). Urchins were also observed spawning during the full moon day in April 2004 in La Parguera (Weil, personal communication). Both observations confirm the results and conclusions of this study.

Sea surface temperatures and salinity fluctuations have been considered by many investigators to be one of the most important triggers that initiate the spawning in many benthic organisms (Bauer 1976). The negative, but significant, correlation found between the GI and the SST might indicate that spawning events in La Parguera could be triggered by temperature changes. In this case, spawning might have been triggered by the increase of SST in spring. Some scientists have argued that spawning also depends on calm waters, increasing the chances, for the larvae to settle near shore (Iliffe et al 1982).

V. Conclusions and Recommendations

- Populations of the black sea urchin *D. antillarum* are recovering in many localities around La Parguera. This recuperation however is slow given the still very low densities measured in this study; Densities still remain low compared to those recorded before 1983.

- There is high variability in the distribution of the urchins within an across reef localities. Mean densities and mean size varied significantly across depth gradients within reefs and across reefs at the same depth interval. Densities of *D. antillarum* were influenced by factors such as depth range, site, and rugosity as reported by Randall (1964) and Weil (1980) Weil et al. (1984).

- Medium size urchins (5 to 8 cm) dominated the population size structure in La Parguera in 2001.

- Larval distribution and successful recruitment in shallow areas in past years coupled with low mortalities due to lack of predation might be the primary reasons for the current status of the populations.

- The current degradation of coral reefs around the Caribbean could have a negative impact on the ability of the black sea urchin population densities to recover as fewer habitats are available for protection.

- *D. antillarum* reproduced all year long in La Parguera, but showed a clear yearly cycle with a peak reproductive activity during the Spring.

- Populations of *Diadema antillarum* in Puerto Rico showed similar reproductive cycles compared to U.S. Virgin Islands, Curacao and Barbados, but there were

differences with the reproductive rhythm in populations in other more southern or northern localities in the wider Caribbean and West Atlantic.

- A complete characterization of the gametogenesis process of the urchin should be done for use as a resource for scientists who are attempting to farm the urchin in the laboratory for re-population programs.

- Monitoring the populations of *D. antillarum* for increase in densities should continue because of its importance role in Caribbean reef communities controlling algal overgrowth and opening space for recruitment of other sessile reef organisms.

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Appendix I. Sea Urchin Gonad Tissue Slide Preparation
  A. Dehydration and clearing of gonad tissue samples:

1. The Dehydration process started by putting a series of samples into different concentrations of Ethanol, and Isopropanol solution (Tissue Dry)

2. Then samples were cleaned in Xylene solution (Tissue Clear III), and finally dipped in Paraplast (Tissue Prep) using a Rotary Tissue Processor (Tissue Tek II, from Sakura)

3. Steps for the dehydration and clearing process; (2 baskets; with ~ 15-20 tissue capsules with samples/basket)

    a. Vase or position 1 and 2 with 70 % Ethanol

    b. Vase or position 3 with 95 % Ethanol

    c. Vase 4,5 and 6 with Tissue Dry (Isopropanol solution)

    d. Vase 7,8 and 9 with tissue Clear (Xylene solution)

    e. Vase 10 and 11 with Paraffin wax (Tissue Prep) at 56 °C

  * Each basket stays 1 hr in each one of the vase or position (1-11)

  B. Embedding of gonad tissue samples:

  1. Tissue Tec Base Molds (size; 32x25x12mm/22x22x12mm) were sprayed with Histo Prep Mold Releasing Agent and placed in the Tissue Tek Tec left side with a temperature of ~ 65 °C.

  2. After the dehydration and the clearing process were complete, samples of one basket were placed in the right side of the Tissue Tek Tec fill with Paraffin wax.

  3. One tissue capsule containing a sample from the right side was choose; then, the Tissue Base Mold, onto witch the sample fits better were selected and filled with

Paraffin wax. After filling the Base Mold with Paraffin wax, the sample selected was placed in the base mold and covered with a labeled (name and date) tissue ring.

4. Step three was repeated with each of the sample capsules. Tissue molds with samples were placed into a freezing plate of the Tissue at ~ 3 °C, until the Paraffin solidified.

5. Embedded samples were stored in the freezer for at least 24h or until they were sectioned.

C. Sectioning of gonad tissue samples:

1. Using a Rotary Microtome (Leitz 1512), longitudinal and cross-sections were obtained (6 - 7μm) from the samples embedded paraffin wax. Then the strip sections were placed in a Warm Bath (Boekel) at 48 ~ 50 °C to be place later in the slides.

2. Strips were picked from the warm bath with a slide that was covered with Albumin Fixative Mayer, to attach the sections to slides. Then, the slides were placed in the slide warmer (Precision) at ~ 48 °C for about 1- 2 hours.

3. Slides were stored at room temperature for at least 24 hours before staining.

D. Staining of the gonad tissue slides:

- Haematoxylin and Eosin were used on the lab to stain the samples.

- Stock Solution = 2.5 g of Haematoxylin + 25 ml Ethanol 95%

Stain solution = 25 ml water + 50 g Aluminum Potassium Sulphate + 500 ml distilled water + 1.25 g Hg2O (Red Mercuric Acid)

Preparation of Haematoxylin:

    a. Add the Alum Potassium Sulphate to the distilled water and bring the solution to boil.

b. Add the Haematoxylin from the stock solution and bring to a boil again

c. Add Hg2O and let it boil slowly for 2 min until the solution turns to a violate color.

d. Cool the water immediately with ice.

e. Let the solution rest for one week in a dark secure place. Store solution in a glass yard.

f. If the solution is too strong and stain the samples to dark, dilute with distilled water.

g. Always filter and decant the small particles in the solution.

Eosin Preparation:

a. 1% Stock solution= Eosin 2.0 g + 40.0ml distilled water

b. Add and dissolve 160.0 ml of Alcohol 95% with the previous solution.

c. Add 600 ml of Alcohol 80% to 200ml of the eosin stock solution (i.e. ratio 1eosin: 3 alcohol). 1

d. Before using the solution, add 0.5 ml of glacial acetic acid to each 100 ml of stain solution and stir.

Staining Process: X= Xylene, % Alcohol and Haem= Haematoxylin

The stations that were set to stain the tissue slides were in the following order:

Station 1: Xy1, Station 2: Xy2, Station 3: Xy3, Station 4: 100 % 1, Station 5: 100 % 2, Station 6: 100 % 3, Station 7: 70 %, Station 8: 30 %, Station 9: H2O, Station 10: Haem, Station 11: H2O, Station 12: Acid Alcohol, Station 13: H2O, Station 14: NH3-H2O, Sation 15: H2O, Station 16: Eosin, Station 17: H2O

Staining steps:

A.   Dip each slide on the station 2 for 3 minutes. All the solutions are contained on glass trays for easy dipping of slides.

B.   Change the slides that will be contained in the slide metal holder to station 3 and leave for 3 minutes

C.   Move slides to station 5 for 1 minute

D.   Move slides to station 6, 7, and 8 one minute on each.

E.   Move slides to station 9 and leave for 3 minutes

F.   Move slides to station 10 and leave samples for 2-20 minutes depending on how dark you want your samples.

G.   After station 10, rinse the slides with running water.

H.   If the slides are stained to dark after you check them under the microscope, go to stations 12, 13 and 14 to clear the slides of any excess of Haematoxylin.

I.   After rinsing the slides with water, go to station 16 and leave the samples in solution for 1-3 minutes.

J.   Rinse samples with running water after station 16.

K.   After the staining has been completed prepared the slides to be dried. Before finishing with the slides go back to station 8 and dip the samples three times in the alcohol 30 %.

L.   Move the samples to station 7 and dip three times.

M.   Continue to deep the samples on every station going backwards from station 7 with 6,5,4,3,2,1.

N.   After dipping the samples in all the stations cover and glue with

cytoseal slide mounting medium and let them dry in a tray.

O   Samples ready to view on the microscope.