

**HERPETOFAUNAL SPECIES COMPOSITION ON THE VIEQUES NATIONAL  
WILDLIFE REFUGE, VIEQUES, PUERTO RICO**

by

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## ABSTRACT

The loss of biodiversity is identified as one of the biggest ecological tragedies of our time and two of the groups most affected by this loss are amphibians and reptiles. A thorough inventory and long term monitoring that provides information on species composition and relative abundance are valuable tools to create a baseline for future assessments of population change in conservation areas. This study addresses and evaluates the herpetofaunal composition, abundance and diversity on the Vieques National Wildlife Refuge (VNWR). The study sites were conducted on the east and west areas of the VNWR, which are located within two different ecological life zones: subtropical dry forest (east area) and subtropical moist forest (west area). Amphibians and reptiles were sampled during eight months between April and November, 2008 using a mixture of three capture techniques: drift fences arrays with pitfall traps, double-ended funnel traps and visual encounter survey (VES) transects. All individuals captured using these techniques were marked with alpha numeric fluorescent tags, which provide individual identification. Abundance, richness and diversity were determined in the study sites on the west and east areas during the wet and dry season. Similarity analyses (ANOSIM) for the sampling areas were conducted to determine the existence of seasonal and spatial patterns in the structure of the herpetofaunal community. A correlation analysis was carried out in order to evaluate the relationship between the environmental variables such as precipitation, relative humidity and temperature and the abundance and richness of species. Population size for *Anolis cristatellus* was calculated using the capture history with models of closed populations and heterogeneous closed populations. An Indicator Species Analysis (ISA) and Similarity Percentage Analysis (SIMPER) were conducted to identify which species characterized the sampling areas, seasons and

sampling periods. A total of 3,248 individuals of 11 species, distributed on 4 amphibian and 7 land reptile species were documented using the three capture techniques. The VES transects were the most efficient capture technique in this study providing greater than 95% of all the documented specimens in the east and west areas. The diversity indices calculated was low for both the east and west areas, although the study sites on the east area provided higher species richness and abundance than study sites on the west area. The similarity analysis indicated no significant differences between sampling areas demonstrating that there is no spatial pattern in the composition among the areas; but indicated a seasonal pattern in the community structure between seasons. *Anolis cristatellus* and *Eleutherodactylus antillensis* were the most abundant species in both areas of the island. *Anolis pulchellus* was the species that characterized the east area during nocturnal sampling, while *Anolis stratulus* characterized the west area during the diurnal sampling. The results of accumulation curves of this study showed a sampling efficiency of 100% for the expected amphibians and reptile species in the west area. The sampling efficiency in the east area documented a 100% of the expected amphibian species and the reptile species presented a 68.6% and 63.2% for the ICE and ACE estimators respectively.

## RESUMEN

La pérdida de biodiversidad es una de las mayores tragedias ecológicas de nuestro tiempo, siendo los anfibios y reptiles dos de los grupos de animales más afectados. Los inventarios robustos y estudios de monitoreo a largo plazo que provean información sobre la composición de las especies y la abundancia relativa, son una herramienta valiosa y línea de base para futuras evaluaciones de los cambios poblacionales en áreas destinadas a la conservación. Este trabajo evaluó la composición, abundancia y diversidad de la comunidad de anfibios y reptiles en el Refugio de Vida Silvestre de Vieques (RVDV). El estudio fue realizado en el área este y oeste del RVDV, los cuales contienen dos zonas de vida ecológicas: bosque seco subtropical (área este) y bosque húmedo subtropical (área oeste). Los muestreos de anfibios y reptiles se realizaron durante ocho meses, entre abril y noviembre de 2008, usando una combinación de tres técnicas de captura: cercas de desvío con trampas de caída, trampas de embudo de doble entrada y transectos de encuentro visual. Todos los especímenes capturados fueron marcados usando etiquetas fluorescentes con códigos alfanuméricos, los cuales asignaron a cada individuo una identificación única. Se determinó la abundancia, riqueza y diversidad en las áreas de estudio ubicadas en el lado este y oeste durante la época seca y húmeda. Se efectuó un análisis de similitud (ANOSIM) para el área este y oeste para establecer la existencia de algún patrón espacial y temporal en la estructura de la comunidad de anfibios y reptiles. El tamaño poblacional para *Anolis cristatellus* fue estimado usando el historial de captura, con modelos de población cerrada y población cerrada con heterogeneidad. Análisis de indicador de especies (ISA) y de porcentaje de similitud (SIMPER) fueron realizados para identificar que especies caracterizaron las áreas de estudio, temporadas y periodos de muestreo. Un análisis de correlación fue realizado para evaluar la relación de

la precipitación, humedad relativa y temperatura con la abundancia y riqueza de especies. Se registraron 3,248 especímenes de 11 especies, distribuidos en 4 anfibios, 7 reptiles terrestres, mediante el uso de las tres técnicas de captura. Los transectos por encuentro visual fue la técnica más eficiente, registrando más del 95% de todos los especímenes en el área este y oeste. Los valores de índices de diversidad fueron bajos para el este y oeste, aunque las áreas de estudio en el lado este generaron mayor abundancia y riqueza de especies en comparación con el lado oeste. El análisis de similitud entre sitios de estudio no registró un patrón espacial en la composición de la comunidad. *Anolis cristatellus* y *Eleutherodactylus antillensis* fueron las especies más abundantes en ambas áreas de la isla. *Anolis pulchellus* fue la especie que caracterizó las áreas de estudio en el este durante la noche, mientras que *Anolis stratulus* caracterizó las áreas de estudio en el oeste durante el día. Los resultados de las curvas de acumulación de especies mostraron un 100% de eficiencia en los muestreos para los anfibios y reptiles esperados en el área oeste; mientras que el área este registró un 100% de eficiencia para las especies de anfibios y un 68.6 % y 63.2 % para los reptiles teniendo en cuenta el estimador ICE y ACE respectivamente.

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## **INTRODUCTION**

### **Background of the Vieques National Wildlife Refuge**

The Vieques National Wildlife Refuge (VNWR) is located in the island municipality of Vieques, Puerto Rico (Appendix A, Figure 1) and is one of the more than 540 refuges in the National Wildlife Refuge System managed by the U.S Fish and Wildlife Refuge Service (USFWS). The VNWR was established in May 1<sup>st</sup> 2001 through the legislated transfer of 3,100 acres of Navy lands located on the western portion of Vieques Island to the Department of Interior, U.S Fish and Wildlife Service. From 1940 until 2001 the U.S Navy used these lands for a variety of purposes including ammunition storage in bunkers, operation of communications facilities, recreational uses, handing and disposal of munitions.

On May 1<sup>st</sup> 2003, Public Law 107-107 amended the Spence Act (Public Law 106-398) and transferred approximately 14,671 acres located in the eastern part of Vieques Island to the Department of the Interior, U.S. Fish and Wildlife Service. This transfer increased the total area of the VNWR to approximately 17,771 acres or 54 percent of the Island (Appendix A, Figure 2) creating the second largest conservation area in Puerto Rico. Since the 1940s, the US Navy and Marine Corps used the farmlands, lagoons, mangrove forest and beaches in the eastern part of Vieques as military training areas. This training facility was known as the Atlantic Fleet Weapons Training Facility (AFWTF) and was divided into the Eastern Maneuver Area (EMA), the Surface Impact Range or the Secondary Impact Area (SIA) and the Live Impact Area (LIA). The EMA was used for such activities as a small arms range, practice minefields, electronic warfare and amphibious landings. The SIA was used for practice shelling from ground artillery positions and from warships offshore. The LIA was the target for the bombs dropped

from jet aircrafts, missiles fired from ships and planes and others types of military maneuvers. In addition to the land areas on Vieques, the surrounding waters were used for an assortment of bombing, missile and artillery exercises. Although the AFWTF was officially closed on April 30, 2003, the contamination is still believed to persist in the area due to the thousands of unexploded ordnance (UXO's) that are still found in soils and marine sediments on approximately 10,000 acres of the eastern tract of the refuge. All these residual explosive compounds, heavy metals, and other chemicals associated with military activities are believed to directly affect others living forms such as invertebrates and vertebrates including humans.

In every refuge, a fauna and flora assessment is required in order to implement appropriate management solutions and to identify species of concern. Faunal inventories and monitoring in VNRW will help determine the effects of anthropogenic disturbances on local wildlife populations. Handling and disposal of many kinds of munitions occur throughout the VNRW. Therefore, there is an imperative need to address baseline studies that document species composition, relative abundance, and distribution in a particular habitat before conducting any restoration or management of degraded habitats.

## **LITERATURE REVIEW**

The loss of biodiversity is identified as one of the biggest tragedies of our time, and two of the groups most affected by this loss are the amphibians and reptiles (Henderson and Powell, 2009). Over the last decades their populations have been declining and some species have disappeared (Stuart et al. 2004). Even though both groups are important components of terrestrial and aquatic ecosystems, they are often ignored (Vitt et al. 1990).

Amphibians and reptiles play a fundamental part of food chains and their populations constitute a high proportion of vertebrates in certain ecosystems (Manzanilla and Péfaur, 2000). Amphibians play essential roles as predators and prey in the ecosystems of the world. Adult amphibians regulate populations of insects that are pests on crops, or that transmit diseases (Dodd, 2010). The tadpoles of many amphibians, as herbivores or filter feeders, play a major role in aquatic ecosystems (Dodd, 2010). Similarly, reptiles can be consumers and prey items helping to balance populations and regulate plant growth as well (Vitt and Caldwell, 2009)

Compared to birds and mammals herpetofaunal species have received little attention in conservation planning. This is because less is known about the structure and biological interactions of members of the herpetological community, which represent the basis for understanding the distribution of species in their habitat (Urbina and Londoño, 2003). However, today herpetofaunal studies that include conservation measures for protected species are being considered as environmental tools for decision makers to declare new protected areas and avoid habitat fragmentation due to urban development. Vitt et al. (1990), Dunson et al. (1992), Blaustein (1994), and Pechmann and Wilbur (1994) all introduce the idea that amphibians serve as “canaries in the coalmine,” or biological indicators, being particularly more susceptible to contamination and environmental changes. This is attributable to several physiological characteristics that include permeable skin, eggs and gills that readily absorb materials from the environment (Duellman and Trueb 1986), and complex life cycles, which include both aquatic and terrestrial life stages (Noble 1931). Reptiles are important because of their position as top carnivores (Vitt et al. 1990). Species of the two groups have been used as indicators of ecosystem health (Gibbons et al 2000).

The richness of amphibians and reptiles in a community depends on factors such as elevation, climate, geographic location and land use (Heatwole, 1982). The species response regarding disturbances of habitat depends on their ability to adapt and population characteristics (Hunter, 1996). Over the past 20 years, many amphibian populations have unexpectedly declined and/or undergone serious reduction ranges (Blaustein and Wake, 1990; Stebbins and Cohen 1995; Houlihan et al. 2000). There are currently 6754 amphibians species reported worldwide (AmphibiaWeb; <http://amphibiaweb.org>). Of these, around 32.5% are now considered endangered, and up to 122 species may be extinct (Stuart et al. 2004). Rapid decline in amphibian populations has been studied in North America (Drost and Fellers 1996; Scout 1993); Caribbean (Hedges 1993; Burrowes et al 2004); Central America (Crump et al 1992; Pounds and Crump 1994; Lips 1998, 1999); South America (Heyer et al 1988; Weygoldt 1989; La Marca and Reinhaller 1991; Coloma 1995; Lynch and Grant 1998) and Australia (Tyler 1991; Laurence et al 1996). Some populations in Puerto Rico have been declining since the 1980's, and these have been linked to a potential synergistic effect between the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) and climate change (Burrowes *et al.*2004). At least four endemic anuran species including the Puerto Rican Crested Toad (*Peltophryne lemur*) and three *Eleutherodactylus* species have experienced population declines (Joglar, 1998; Joglar and Burrowes, 1996) and presumably extinction such as *E. eneidae*, *E. jasperi*, *E. karlschmidti* as also the northern population of *Peltophryne lemur*. In addition, another six *Eleutherodactylus* species (*E. locustus*, *E. richmondi*, *E. gryllus*, *E. wightmanae*, *E. portoricensis* and *E. coqui*) are declining in parts of El Yunque, one of the best protected forests on the island (Burrowes *et al.*2004).

Reptile species also present population declines, although these declines have received less attention even when documented (Gibbons et al. 2000, Reaser 2000). The

secretive nature of many reptiles often combined with comparatively large home ranges, low population densities, and rareness of congregational behavior, make documenting population trends very difficult (Gibbons et al. 2000). There are currently 8734 reptiles species reported worldwide (Uetz, 2008). Of these 22% are endangered (Baillie et al 2004) and 21 species recently extinct (Whittaker and Fernandez-Palacios 2007). Some reasons to explain worldwide declines include continuous habitat loss and degradation, competition and predation from introduced species, environmental pollution, diseases, parasitism and global climate change. In Puerto Rico, eleven reptilian species are currently protected as threatened or endangered (Joglar et al., 2007). The Puerto Rican boa (*Epicrates inornatus*) has experienced population decrease. Human impact, via habitat loss and fragmentation and killing because of innate fear or ignorance, and mongoose predation are main factors affecting the species (Reagan and Zucca 1982). The Puerto Rican Freshwater turtle (*Trachemys s. stejnegeri*) populations are almost unknown and early studies indicated potential threats to natural populations (Joglar et al 2007). The threats include intense egg predation by the exotic Indian mongoose (*Herpestes javanicus*) affecting the reproductive success and recruitment of early life stages and the habitat alteration and establishment of exotic freshwater turtles (*Trachemys scripta elegans*) in natural ecosystems inhabited by the Puerto Rican freshwater turtle (Joglar et al 2007) and perhaps even hybridization with this introduced species.

Studies with the Mona Island Iguana (*Cyclura stejnegeri*) estimate population densities ranging from 0.33 individuals/ha (Wiewandt, 1977) to  $0.96 \pm 0.47$  individuals/ha (Pérez-Buitrago and Sabat, 2000), densities that are very low for this group (Iverson, 1977). The main threats to these endangered iguanas are invasive mammals such feral pigs and goats that predate the nesting sites and compete for vegetation.

Islands habitats comprise some 3% of the earth's land area, but harbor 20% of all bird, reptile and plant species. Extinction rates are also exponentially greater on islands: 95% of bird, 95% of reptile, 69% of mammal and 68% of plants extinctions have occurred on islands, most of these extinctions caused by invasive species such as feral goats and rats. The Puerto Rican island shelf (Heatwole and MacKenzie, 1966) comprises 70 reptile and 27 amphibian species (Hedges, 2010); Puerto Rico has the highest number of native species of amphibians per area in comparison with the other Greater Antillean islands (Joglar et al 2007). The herpetofauna of Puerto Rico and the adjacent islands have been studied since the early 1800s when Merrem (1820) described the first endemic species, Puerto Rico giant anole; *Anolis cuvieri*. This discovery gave way to the knowledge of species richness throughout the island of Puerto Rico and adjacent islands including Vieques.

Vieques Island is located approximately seven miles east from the Main Island and 20 miles southwest of St. Thomas, U.S. Virgin Islands. It has a surface of about 51 squares miles or 33, 120 acres (13,403 ha) and is located between 18°10'N and 18°05'N latitude, and 65°35'W and 65°16'W longitude. The amphibians and land reptiles on Vieques have been studied since 1900s when Stejneger (1904) published the first list of amphibians and land reptiles from Vieques including eleven species, two frogs and nine land reptiles.

Schmidt (1928) removed *Typhlops jamaicensis* from Stejneger's (1904) list. Grant (1932) added six new species and removed *Sphaerodactylus grandisquamis* or *S. macrolepis* bringing the total to 15 reported species. Thomas and Schwartz (1966) in taxonomical studies reevaluated and added two new subspecies *S. nicholsi townsendi* formerly *S. townsendi* and *S. macrolepis inigo* formerly *S. danforthi*. Rivero (1978) added *Rhinella marina* (formerly *Bufo marinus*) and *Amphisbaena caeca* and removed five

species from the list published by Grant (1932) leaving a total of 12 species. Saliva (1994) based in Grant (1932) and Rivero (1978) lists 15 amphibians and land reptiles. Rivero (1998) included *Eleutherodactylus coqui*, *Mabuya sloanii*, *Iguana iguana* and *Anolis roosevelti*; for a total of 16 species. The comprehensive conservation plan and environmental impact statement (CCP) for the VNWR (U.S Fish and Wildlife Service, 2007) reported 31 species; these species include 22 land reptiles, 5 amphibians and 4 marine sea turtles species (three nest on Vieques). All previous reports have been limited to document presence or absence of species, but no herpetological studies evaluate species composition, relative abundance and distribution in Vieques Island. Due to the pressure of land use in Vieques, surveys and monitoring programs have become a priority for the VNWR in order to implement management and restoration practices in areas that for years were used for military training activities.

On Vieques there are four protected areas dedicated to conservation. Two major parcels or tracts (one in the east and one in the west) are protected and administrated by the USFWS and constitutes a total area of 17,769 acres (7,191 ha) or approximately 54 percent of the Vieques island. The others two parcels with an area of 823 acres (333 ha) and 1165 acres (471 ha) are protected and administrated by Puerto Rico Conservation Trust and the Puerto Rico Department of Natural Resources (DNRA) respectively.

A thorough inventory that provides information on species composition and relative abundance based on a repeatable sampling scheme is a valuable tool and baseline for future assessments of population change (Lips et al 2001). For this reason floral and faunal surveys and monitoring have become a priority for the managers of these conservation areas, especially in the areas that were used for military training activities.

This study will assess the terrestrial herpetofaunal species composition, relative abundance, and distribution in the east and west tracts on the VNWR.

## **OBJECTIVES**

The objectives of this study are to:

1. Quantify the relative abundance, distribution, and diversity of amphibians and land reptiles in the study areas on Vieques National Wildlife Refuge.
2. Estimate the change in richness, relative abundance and composition of land amphibians and land reptiles between the wet and dry seasons.
3. Determine the influence of a disturbance on the abundance and richness of amphibians and land reptiles, making a comparative analysis of the study areas on the Vieques National Wildlife Refuge.

## MATERIALS AND METHODS

### *Study areas*

The study sites are located within two ecological life zones (Ewel and Whitmore, 1973), the subtropical dry forest (two-thirds of the island) and the subtropical moist forest. The study was conducted on the east and west areas of the VNWR which includes the Monte Pirata Area and the south side of Laguna Kiani in the west area and Puerto Ferro Peninsula, Water tank road, Malojillo Hill Area, the former Live Impact Area (LIA) south access road mile mark 4.7, the Carenero Area (former aircraft approach road to target # 2), and the first north access road before gate 3 in the east area. Because most of the eastern portion of the VNWR is closed to the public, the access and establishment of the study areas were limited by the U.S Navy. (Appendix A, Figure 3).

On Vieques, these two ecological life zones are covered by various plant communities with different structures and compositions (U.S Fish and Wildlife, 2007). Two vegetation types comprise the study areas, dry forest and shrub at the eastern tract and mixed evergreen-deciduous forest at the western tract.

The west area comprises 710 acres and contains the highest peak on the island (18.093150° north / 65.551210° west) Monte Pirata, with an elevation of 987 feet (301 mt) above sea level. This area is characterized by a young and mature secondary forest and is the most diversified and undisturbed association with the greatest number of plant species present. The young secondary forest is found on the bases of hills as well as some smaller ravines with an average of tree height of 8-10 meters. The species composition consists of the slender fan palm (*Coccothrinax alta*), Almacigo tree (*Bursera simaruba*), *Eugenia biflora*, *Inga laurina*, *Coccoloba venosa*, *Guapira fragrance*, *Myrciria myrtifolia*, *Acalypha berteriana*, *Malpighia fucata* and *Casearia guianensis*. The

roadside vegetation leading up to Monte Pirata is quite disturbed and supports various weedy species such as *Leucaena leucocephala*, *Cordia polycephala* and *Mimosa ceratonia*.

The study sites at the east area are within the plant communities of dry forest and shrub. This vegetation has been greatly modified from their original vegetative cover. The areas are characterized as dense, dry, spiny woodland and shrub. The Malojillo hill area, the former Secondary Impact Area (SIA) south access road at mile mark 4.7, and the Carenero Area (former aircraft approach road to target # 2) are characterized by exotic and altered vegetation called mesquite savanna and involves the *Prosopis/Bucida* woodland alliance including *Acacia macracantha*, *Acacia farnesiana* and *Leucaena leucocephala* with *Panicum maximum* or *Chloris barbata*- *Dichanthium annulatum* dominating the herbaceous layer. The Puerto Ferro area is best represented on the limestone bedrock and is composed mainly by native dry forest vegetation. Common species include *Coccoloba* spp., *Pisonia subcordata*, *Krugiodendron ferreum*, *Crossopetalum rhacoma*, *Bourreria succulenta*, *Gymnanthes lucida*, *Rauvolfia nitida*, and *Bursera simaruba*. The Malojillo area located on northern hilltops is composed mainly by *Thrinax morrisii* and *Bursera simaruba*.

### ***Capture Techniques***

Amphibians and reptiles were sampled using a mixture of three capture techniques commonly used in herpetofaunal studies. The use of several capture techniques is due to evasive nature of reptiles and amphibians and the diversity of amphibians and land reptiles reported previously on Vieques as well as the different perches and activity periods for each group. The capture techniques were drift fences arrays, funnel traps and visual encounter survey (VES) transects, details of each method in Bury and Corn (1987),

Heyer et al (1994) and Lips et al (2001) with modifications made due to the study areas and the topography. Several kinds of materials have been used for drift fences in pitfall array designs; in this study the drift fences were constructed of silt fence similar to that used to control sediment runoff at construction sites (Enge, 2001). Due to the different substrates found in the study sites, the use of silt fence instead of aluminum galvanized flashing or fiberglass screen was the most appropriate material with a relatively easy installation. The silt fence was attached to wooden stakes which were driven into the ground and canals were made in the ground to bury the silt fence to avoid space between the fence and the ground surface. The design for arrays of drift fence was arranged using four arms of silt fence and five pitfall plastic buckets (Appendix A, Figure 4a,b). The pitfall buckets were placed at the ends of the four arms and at the center where the four fences meet at a distance of 10 m (Appendix A, Figure 4c). Each pitfall bucket (13 inches height and 9.5 inches width) of 5 gallons was buried and four holes of 1/8 inch were drilled in the bottom of each bucket to prevent the accumulation of water. Leaf litter was placed at the bottom of the buckets to maintain humidity and create refuges that will help to avoid desiccation of the captured animals (Appendix A, Figure 5a). The lids of the buckets were used to create shade inside the bucket and to reduce the possibility of desiccation and escape of captured animals, these lids were attached to strong wire forming two legs 3.5 inches high (Appendix A, Figure 5b).

Double ended funnel traps were another capture apparatus used in this study. The traps were installed in each arm of the drift fence on one side. The traps were constructed of aluminum screening (Appendix A, Figure 6a,b). The pieces were rolled and held together by staples and aluminum utility wire forming a cylinder.

The funnels were made with two pieces of aluminum screening folding the sides and stapling through the overlapped sections. To set the funnel on the cylinder, the edges

were folded and held with  $\frac{3}{4}$  inches binder clips (8 per funnel). This allowed easy removal of captured animals. The body of the funnel trap measured 36 inches in length and each funnel had an outside diameter of 10 inches (25 cm) and an inner-opening diameter of 3.5 inches (8.9 cm). Before the activation of these traps, the site was cleared removing all branches and leaves and the space between the trap and the drift fence was cleaned and the soil stabilized to prevent the escape of organisms through gaps in the soil or between the fence and trap.

Twelve drift fences arrays and 48 funnel traps were established in the study sites chosen on the west and east areas of the island. On Monte Pirata area, three drift fence arrays were placed in site 1 and site 2 for a total of six arrays on the west area (Appendix A, Figure 7). The six drift fence arrays were established in inclined areas due to the mountain topography characterized by hillsides, deep ravines, hilltops and ridges. On the east area of the island, another six drift fences arrays were established in three sites, in this case two drift fences arrays were placed on each site, Puerto Ferro (site 1), Malojillo hill (site 2) and former Surface Impact Area (SIA) (site 3) (Appendix A, Figure 8), with the purpose of sampling the heterogeneity of habitats present. These areas are characterized by flat surfaces dominated by small trees, shrubs and grassy areas.

VES transects were the third survey method used in this study to measure species composition and relative abundance. VES transects were 125 meters long and 6 meters wide. Along transects reflective tape was attached to the trunks or branches for better orientation during the nightly surveys. The purpose of including VES transects in the study was to cover areas that were not surveyed by the drift fences arrays and funnel traps to increase the sample size of captured animals. The transects were walked slowly and carefully searching the canopy trees, branches, leaf litter, and space under rocks, log retreats or under artificial retreats such as cement structures, piece of wood or boards.

On Monte Pirata area, three VES transects were established. One transect (site 3) was 60 m from the drift fence array in the site 1, near an intermittent creek situated northeast of the telecommunication towers. The second transect (site 4) was located 50 m from the drift fence array situated northwest of the telecommunication towers, 320 m away from the first. The third transect (site 5) was established at the base of Monte Pirata on the north side near lands belonging to the Municipality of Vieques (Appendix A, Figure 9). Three additional transects were established on the east area of the island. One transect was located near the Camp Garcia facilities (site 4), the second was installed on the north side of the south access road (site 5), and the third transect was sited in the Carenero Area (former aircraft approach road to target # 2) and the first north access road before gate 3 (site 6) (Appendix A, Figure 10).

### ***Sampling Methodology***

Twenty two samplings were conducted between April and November 2008, including the dry and wet seasons. Each sampling consisted of searches performed both daily and nightly during three consecutive days; each study site in the east and west area was sampled during the morning and night, for a total of 11 samplings per study area. Due to weather conditions and accessibility to the study sites, some samplings were done consecutively in the same areas but with at least a four day period of separation.

At the beginning of each sampling, the pitfall and double-ended funnel traps were left open until the third sampling day in the morning when they were closed until the next sampling session. The pitfalls were inspected twice per day every 12 hours with a total sampling effort of 72 hours/person in the sites of drift fences arrays. Every sampling operated six drift fences arrays with 30 pitfall and 24 double-ended funnel traps opened,

covering 2,400 m<sup>2</sup> in the drift fence areas, and 2,250 m<sup>2</sup> in the VES transect areas, for a total sampling area of 4,650 m<sup>2</sup>.

Each VES transect was walked ones at day, ones at night for 1.5 hr each during the sampling period. Each transect was walked 22 sampling sessions for a total sampling effort of 648 hours/person in transects sites.

### ***Species Documentation***

Most of the individuals captured during the study period were identified in the field. The individuals that were not identified in the field were photographed and transported to the refuge station for further identification using the key described by Rivero (1998). Data sheets for the searches were modified from Lips et al (2001). The animals were captured by hand or using a panfish pole with a noose at the end. For each animal captured, the measurements of the snout vent length (SVL) (cm) and the weight (g) were obtained. The technique used for individual identification was an alpha numeric fluorescent tag (Northwest Marine Technology Inc, Shaw Island Washington). This identification technique was used only for anoles and frogs species. These alpha numeric fluorescent tags are an alternate method to identify amphibians and reptiles and are made of a flexible material from a medical grade, silicone- based elastomer, and they do not irritate the tissue at the implant site. These tags have been used extensively on fish (Frennete and Bryant 1996) and amphibians (Buchan et al 2005) but minimal work has been done on reptiles, especially lizards.

Every tag has a unique alpha numeric identifier composed of a letter (A-Z) and number (00-99) (Appendix A, Figure 11a). The codes are made with fluorescent colors (e.g., orange, green, red, black) with various background colors (e.g., red, orange, black, yellow) and are available in two sizes, standard (1.0 mm x 2.5 mm) and large (1.5 mm x

3.5 mm). For this study black codes on orange background was the combination most used. The animals were tagged by making an incision with the injector (provided by Northwest Marine Technology) without applying anesthesia (Appendix A, Figure 11b). The tags in anoles species were placed under the subcutaneous layer on the ventral side of a hind limb, injecting the tag completely (Appendix A, Figure 11c). The tags in frog species were placed under the subcutaneous layer on the dorsal hind limb. When the animal had dark skin we used a UV light to read the tag (Appendix A, Figure 11d), but in most of the recaptured animals the tags were read without the UV light. After tag injection an antibacterial cream was applied to the incision zone to prevent infection. Post-injection sterilization was used and consisted of cleaning the injector with alcohol before using it on another animal or storing it. All tagged animals were released at the capture point (Appendix A, Figure 11e).

A capture-recapture study was conducted with the marked individuals to estimate the population size of *Anolis cristatellus*. The other marked species did not provide adequate data in terms of capture history to estimate population size in MARK program version 5.0 (White y Burnham 1999).

### ***Environmental variables***

Climatological data such as temperature and relative humidity were recorded using a Kestrel 3000 Pocket Weather Station in the study sites. These data were recorded at the beginning of every sampling period. Precipitation data was obtained from a weather station located in Vieques property of SE Regional Climate Center, Columbia, SC. The weather station is located 8.7 km from the east study sites and 14.5 km from the west study sites. The environmental variables were examined in order to know if the

composition and abundance of amphibians and reptiles vary with climatological measures in the two distinct habitat types.

### ***Statistical Data Analysis***

The total abundance was determined from the number of captures from each transect and drift fence array in the entire sites. The relative abundance was obtained by dividing frequency of the species by sampling effort. The mean relative abundance with confidence interval was used to compare the abundance of each species in the study areas. The relative abundances were significantly different statistically when the overlap between intervals was less than 25% (Cumming *et al.* 2007). The species diversity of amphibians and reptiles was calculated based on the species richness in the east and west area of the island. Diversity indices as Shannon-Wiener (H), Simpson ( $\lambda$ ) and Margalef richness (d), were calculated using PRIMER 6.0 (Clarke and Gorley, 2006). The descriptions of them are in Villarreal et al (2004). Similarity analysis (ANOSIM) were carried out to determine difference in the herpetofaunal composition in the east and west areas and between the dry and wet sampling seasons. A similarity percentage analysis (SIMPER) was conducted to identify which species characterized the sampling sites and seasons, based in the abundance of each herpetofaunal species. The species that provided greater than 10% to the total abundance were selected to characterize the sampling sites and seasons. In addition, species accumulation curves based in the species richness estimators ICE (Incidence-based Coverage Estimator) and ACE (Abundance-base Coverage Estimator) were obtained to estimate the sampling representativeness in the study areas, using EstimateS 6.0b1 (Colwell 2000).

The total population size was only estimated for *Anolis cristatellus* because sufficient recapture data to estimate the population size was obtained. The capture history

was analyzed using the MARK 5.0 (White and Burnham 1999) with models of closed populations and heterogeneous closed populations. For the *Anolis cristatellus* capture history in the east and west study sites, predefined models in MARK were used and others models were conducted based in the environmental characteristics of each sampling period with the purpose to determine if the environmental variables affected the capture probabilities (p) and the recapture of individuals (c). The models evaluated were:

**p(.) ≠ c(.):** capture and recapture probability are different, but constant over time.

**p(.) = c(.):** Equal capture and recapture probability but constant over time.

**p(t) ≠ c(t):** capture and recapture probability are different and time dependant.

**p(t) = c(t):** Equal capture and recapture probability and time dependant.

**p(t) ≠ c(.):** capture and recapture probability time dependant and constant respectively.

**p(.) ≠ c(t):** capture and recapture probability constant and time dependant respectively.

**Mh:** Heterogeneous closed population's model, capture histories divided in two groups with different capture probabilities.

**p(environment) ≠ c(environment):** capture probabilities different for the recaptures and different for the dry season in comparison with wet season, but equal within each season.

**p(environment) = c(environment):** capture probabilities equals to the recaptures and different in the dry season in comparison with wet season, but equal within each season.

**p(environment) ≠ c(.):** capture probabilities different among dry and wet season, recapture probabilities constant over time.

**p(environment) ≠ c(t):** capture probabilities different among dry and wet season recapture probabilities time dependant.

**P(.) ≠ c(environment):** capture probabilities constant over time and recapture probabilities different among wet and dry season.

**P(t) ≠ c(environment):** capture probabilities changing over time and recapture probabilities different among wet and dry season.

The best model was chosen based on the low Akaike's Information Criterion (AIC) value (Cooch and White 2008) and on the  $\Delta AICc$  proportion test and the AICc Weight.

Environmental variables such as precipitation, relative humidity and temperature were analyzed with a correlation analysis using PAST 1.63; in order to detect as relations with the abundance and richness of species in the study sites. A Multi-response Permutation Procedure (MRPP) was conducted to compare all east pairwise groups and sampling periods (AM/PM) with all west pairwise groups and sampling periods (AM/PM). An Indicator Species Analysis (ISA) ISA was used to determine which species can distinguish the study areas and the sampling periods. The last two analyses were obtained using PCORD 5 (McCune and Mefford., 1997).

## RESULTS

### *East area*

During eight months of field sampling, a total of 1,879 individuals of 11 land species were documented throughout the six study sites located on the east area of the Vieques National Wildlife Refuge (Appendix B, Table 1). From these individuals, 1,860 (98.98%) were recorded via visual encounter survey transects and 19 individuals (1.02%) were documented using the drift fence arrays with pitfall traps and double ended funnel traps. The visual encounter transect located in site 5 produced the highest abundance with 811 (43.16%) individuals; the other visual encounter transects located in site 4 and 6 recorded 423 (22.51%) and 626 (33.32%) individuals respectively. At sites 1, 2 and 3 the drift fence arrays produced 6, 7 and 6 individuals respectively (1.02%). The dry and wet seasons were classified according the precipitation regimens on the Vieques island during 2008 (Figure 1). During the dry season (April to July) the highest abundance (699 individuals) was observed in June and the lowest abundance (156 individuals) in July (Figure 2).

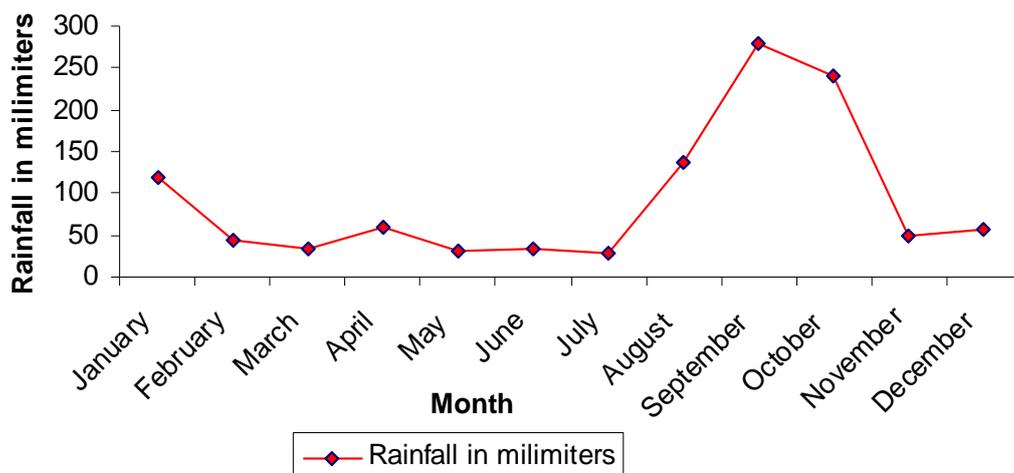


Figure 1. Total precipitation by month on Vieques Island, Puerto Rico, 2008. (Taken from Western Regional Climate Center)

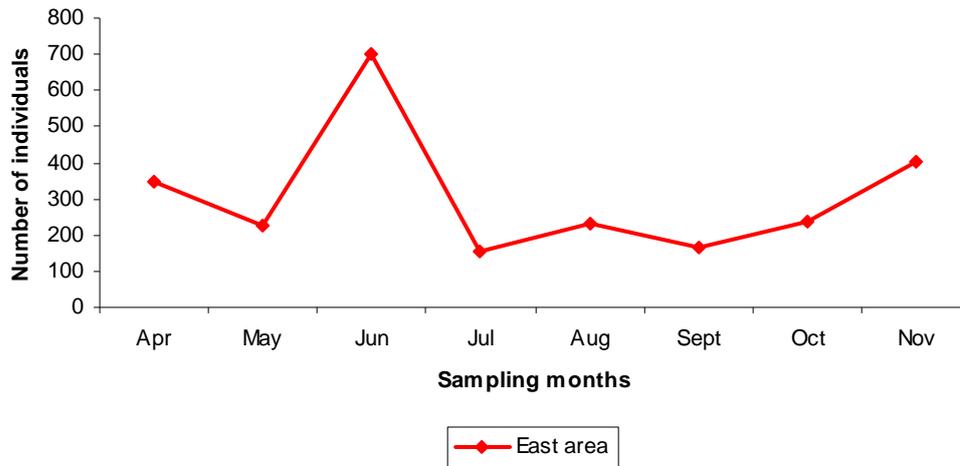


Figure 2. Monthly variation of the abundance of individuals at the east area of the Vieques National Wildlife Refuge.

The three most abundant species over all the six study sites on the eastern area were *Eleutherodactylus antillensis*, *Anolis pulchellus* and *Anolis cristatellus*. These three species represented 8.30%, 24.85%, and 48.48%, respectively of all documented individuals in the visual encounter transect, drift fence arrays and double ended funnel traps. The reptile species provided most of the herpetofaunal abundance in the east area with 1,506 individuals of 7 species providing 80.15% of all herpetofaunal abundance species (Figure 3). Four of the seven species reached maximum abundance in June and the three remaining species stayed with a constant abundance over the entire study (Figure 4).

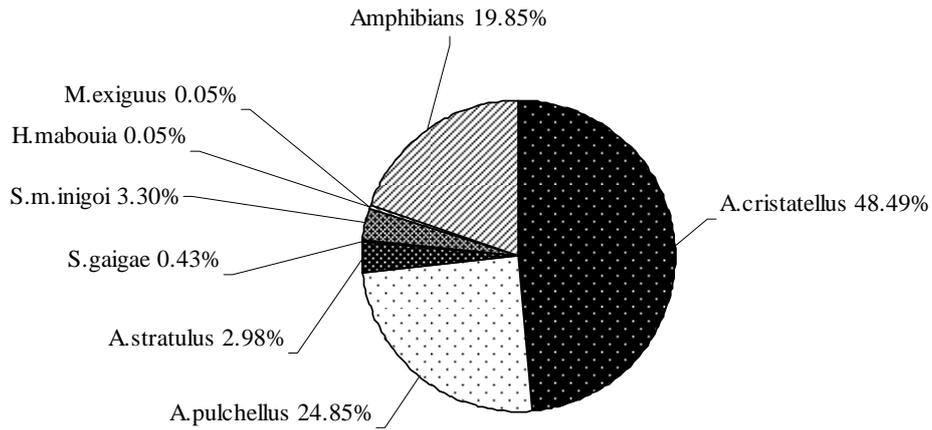


Figure 3. Abundance of reptile species in comparison with amphibians group at the east area of the Vieques National Wildlife Refuge.

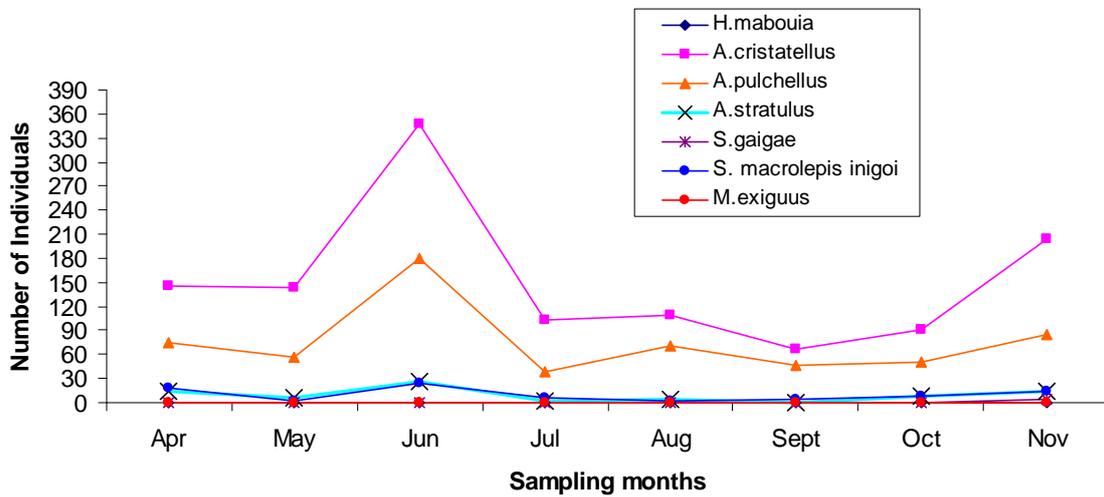


Figure 4. Monthly variation of the abundance of reptile species at the east area of the Vieques National Wildlife Refuge.

In terms of amphibian species, 373 individuals of 4 species were registered and constitute 19.85% of the herpetofaunal abundance species in the east area of the island (Figure 5).

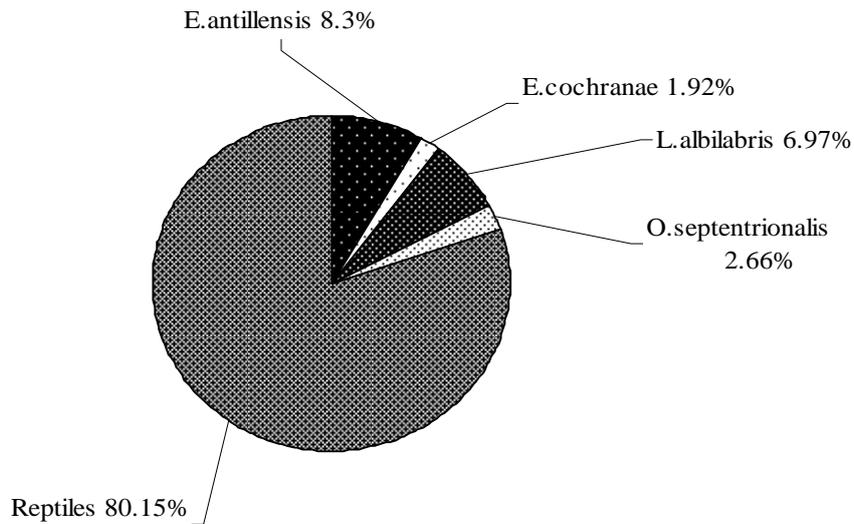


Figure 5. Abundance of amphibian species in comparison with reptiles group at the east area of Vieques National Wildlife Refuge.

*Eletherodactylus antillensis*, *Elutherodactylus cochranæ* reached highest abundance on April and June; *Leptodactylus albilabris* reached highest abundance in April, June and October; and *Osteopilus septentrionalis* stayed with constant abundance values over the sampling months (Figure 6).

A total of 378 individuals were marked using the visible implant alphanumeric tags. These animals were distributed in 6 species. During the sampling period, 196 (51.85%) individuals of four species were recaptured during the eight months of field sampling. *Anolis cristatellus* registered 251 marked individuals and 136 recaptured (54.18%) and was the most marked and recaptured species on the east area of the island, followed by *Anolis pulchellus* with 99 individuals marked and 44 recaptured (44.44%), *Eletherodactylus antillensis* with 15 individuals marked and 11 recaptured (73.33%), and *Anolis stratulus* with 10 individuals marked and 6 recaptured (60%). *Leptodactylus albilabris* and *Magliophis exiguus* had 2 and 1 marked individuals respectively but no recaptures were obtained. Mean captures per sample were greater during the wet season

for *Anolis cristatellus* ( $\bar{x} = 40.3$ ,  $SE = 4.21$ ), *Anolis pulchellus* ( $\bar{x} = 17$ ,  $SE = 1.34$ ), *Eleutherodactylus antillensis* ( $\bar{x} = 3.17$ ,  $SE = 0.90$ ) and *Anolis stratulus* ( $\bar{x} = 2.17$ ,  $SE = 1.07$ ).

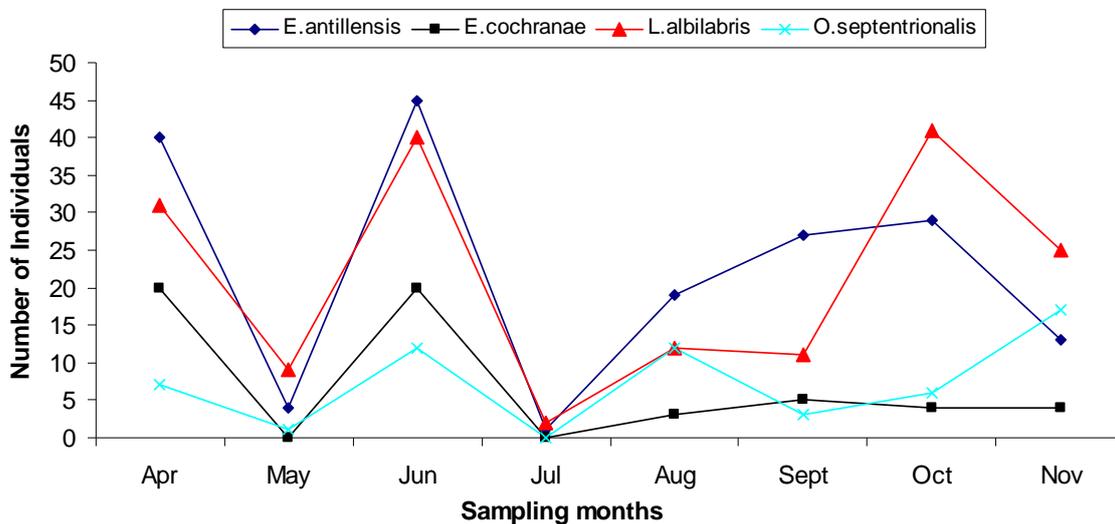


Figure 6. Monthly variation of the abundance of the amphibian species at the east area of the Vieques National Wildlife Refuge.

### **West area**

During eight months of field sampling 1,369 individuals of 8 species were documented throughout the five study sites located on the western area of the Vieques National Wildlife Refuge (Appendix B, Table 2). As on the east area, the most efficient capture technique was the visual encounter transects, which provided 1,339 (97.81%) of all the individuals documented. On the other hand, the drift fence arrays with pitfall traps and double ended funnel traps captured only 30 individuals (2.19%). The transects in sites 4 and 3 provided the highest abundance with 314 (36.46%) and 299 (34.72%) individuals respectively during the dry season. In the wet season, site 3 captured 267 (52.56%) and site 4 captured 217 (42.72%) individuals. Site 5, where another transect was established produced 231 (26.83%) individuals in the dry season and 11 (2.16%) individuals in the wet season, making this transect the less abundant in comparison with

the other transects. Species richness was highest on site 4 (8 species), with one additional species than site 3. Sites 1 and 2 with the drift fence arrays with pitfalls and the double ended funnel traps provided 8 (0.93%) and 9 (1.04%) individuals during the dry season and 7 (0.81%) and 6 (0.69%) individuals in the wet season. Species richness on site 1 and 2 was the same with 4 species each. The dry season provided the higher abundance with 861 individuals. Of these capture, 345 were obtained on June. The abundance on wet season (August to November) was 509 individuals and was lower compared to the dry season, being August the month with highest abundance (183 individuals) (Figure 7).

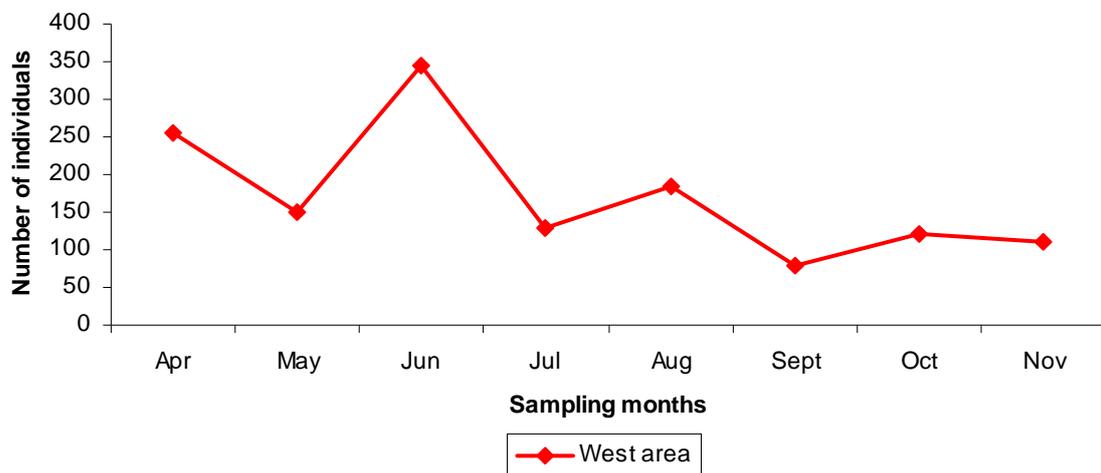


Figure 7. Monthly variation of the abundance of individuals at the west area of the Vieques National Wildlife Refuge.

During the entire study the most abundant species on the west area of the Refuge were *Anolis cristatellus* (636 individuals), *Sphaerodactylus gaigeae* (192 individuals) and *Eleutherodactylus antillensis* (178 individuals) and *Anolis stratulus* (151 individuals). These four species respectively represented the 46.45%, 14.02%, 13%, and 11% of all individuals registered in the five sites (Figure 8). *Anolis cristatellus*, *Anolis stratulus*, *Sphaerodactylus macrolepis inigoi* and *Sphaerodactylus gaigeae* showed the highest

abundance in June, while the remaining species did not present significant variations over the sampling period (Figure 9).

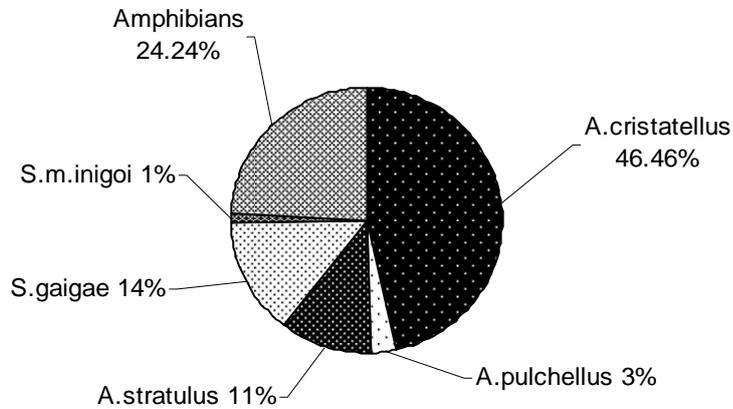


Figure 8. Abundance of reptile species in comparison with amphibians group at the west area of Vieques National Wildlife Refuge

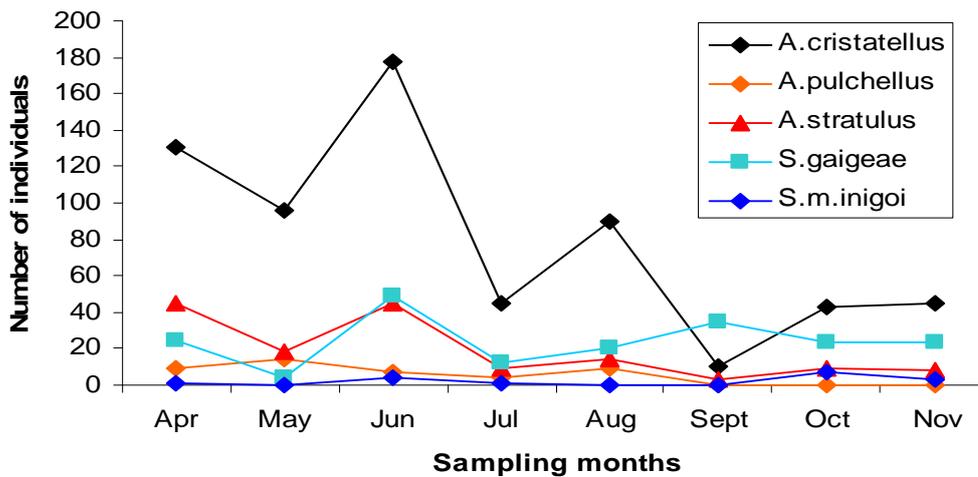


Figure 9. Monthly variation of the abundance of the reptile species at the west area of the Vieques National Wildlife Refuge.

In terms of amphibian species, 332 individuals of 3 species, were registered and constitute 24.24% of the herpetofaunal species in the west area of the island from all the individuals documented using visual encounter survey and drift fence arrays and double ended funnel traps (Figure 10).

Alphanumeric tags were implanted on 247 individuals distributed on 2 amphibian species and three reptile species. A total of 146 (59%) individuals were recaptured.

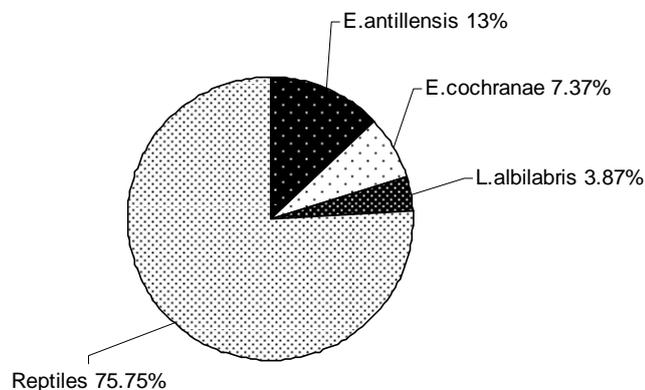


Figure 10. Abundance of amphibian species in comparison with reptiles group at the west area of Vieques National Wildlife Refuge.

*Anolis cristatellus* was the species with higher number of marked individuals. A total of 205 were marked and 127 recaptured (61.95%), followed by *Eleutherodactylus antillensis* 31 individuals marked and 13 recaptured (41.93%); *Anolis stratulus* 8 individuals marked and 6 recaptured (75%); *Anolis pulchellus* and *Eleutherodactylus cochranae* with 2 and 1 marked individuals respectively but no recaptures were obtained from these two species.

Mean captures per sample were greater during the dry season for *Anolis cristatellus* ( $x = 29.7$ ,  $SE = 6.39$ ). On the other hand, for *Eleutherodactylus antillensis* ( $x = 6.4$ ,  $SE = 1.74$ ) and *Anolis stratulus* ( $x = 1.8$ ,  $SE = 0.72$ ) captures per sample were greater during the wet season.

The total species documented in the entire study was 11 species; 4 amphibians and 7 land reptiles. This study was conducted in upland habitats excluding the coastal habitat, where four sea turtles species have been documented nesting and foraging in several beaches around Vieques Island. The study sites on the east area showed higher species

richness than study sites on the west area. The Shannon diversity index both for frogs and for lizards was low for both the east and west areas with no significant differences ( $P = 0.56$ ,  $P = 1.0$ ) (Table 1).

The dominance was relatively low and similar for both areas according the Simpson index, as dominance increases, diversity decreases (Magurran 2004), and hence the low diversity obtained cannot be fully explained by the dominance of a few species in the recorded herpetofaunal.

Table 1. Diversity index values of the Shannon-Wiener (H), Simpson ( $\lambda$ ) and Margalef (d) for amphibians and reptiles during the seasons sampling at the west and east areas of the Vieques National Wildlife Refuge.

Order	Sampling area	Shannon-Wiener	T Hutchenson (df)	Simpson	Margalef
Reptilia	East	0.96	0.56 (47)	0.46	1.65
	West	1.09		0.43	1.28
Amphibia	East	1.23	1.0 (16)	0.32	1.34
	West	0.99		0.40	1.01

The reptile species abundance was higher in the study sites at the east area than the west area (Figure 11a). In general, there was statistically significant difference between the abundance of each species in each area because there was less than 25% overlap in the confidence intervals. Abundance of amphibian species did not show a difference between the study areas (Figure 11b). Only one species, *Osteopilus septentrionalis*, was not recorded at the west area.

Similarity analysis (ANOSIM) indicated no significant differences between sampling sites at the east and west areas ( $R = 0.048$ ;  $P = 0.20\%$ ), demonstrating that there is no spatial pattern in structure and composition among the areas. The ANOSIM

among seasons dry and wet indicated a seasonal pattern in the community structure, representing significant statistical differences ( $R = 0.566$ ;  $P = 0.01$ ).

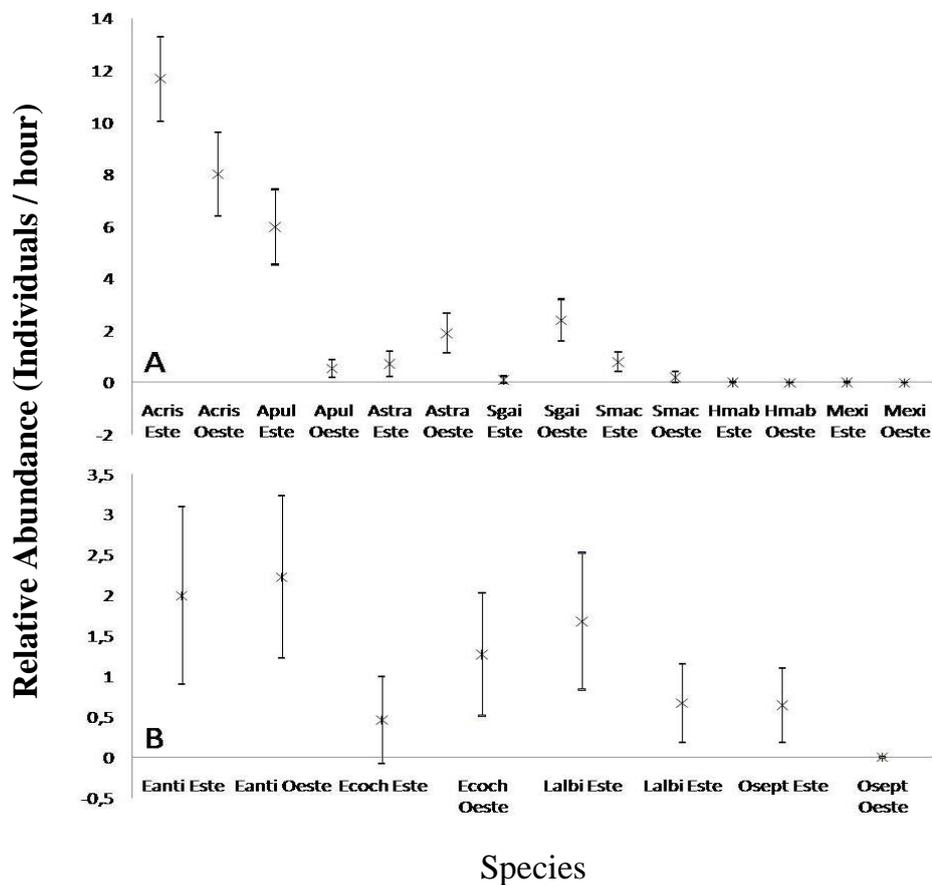


Figure 11. Relative abundance for reptile (A) and amphibian (B) species, documented at Vieques National Wildlife Refuge. Acris = *A. cristatellus*; Apul = *A. pulchellus*; Astra = *A. stratulus*; Sgai = *S. gaigeae*; Smac = *S. macrolepis*; Hmab = *H. mabouia*; Mexi = *M. exiguss*; Eanti = *E. antillensis*; Ecoch = *E. cochranae*; Lalbi = *L. albilabris*; Osept = *O. septentrionalis*. Asterisk in vertical bar indicate means and horizontal bars indicate confidence intervals.

Differences were observed among east-dry/west-dry; east-dry/west-wet; east-wet/west-dry; east-wet/west-wet and west-dry/west-wet; the only pairwise test group where there was no significant difference was east-dry/east-wet (Table 2). Similarity percentage (SIMPER) analysis was used to determine which species were most responsible for the difference in the total abundance between areas and seasons. Two

species on the east and west areas (*Eleutherodactylus antillensis* and *Anolis cristatellus*) provided a 58% and 64% respectively of the total abundance for the east and west areas.

Table 2 ANOSIM analysis among dry and wet season for the herpetofaunal community structure at the east and west areas of the Vieques National Wildlife Refuge.

Pairwise Tests-Groups Comparison	R statistic	Significance level (%)
East-Dry / East-Wet	0.083	20.6
East-Dry / West-Dry	0.797	0.2
East-Dry / West-Wet	0.596	0.8
East-Wet / West-Dry	0.891	0.2
East-Wet / West-Wet	0.691	0.2
West-Dry / West-Wet	0.283	2.6

*Anolis pulchellus* and *Anolis stratulus* were the species with the lower percentage contribution (13.54 and 17.88%) and are indicator species that distinguish the east and west areas respectively (Table 3).

Table 3. SIMPER analysis of indicator species at east and west areas of the Vieques National Wildlife Refuge.

Species	Contribution (%)	
	East	West
<i>Eleutherodactylus antillensis</i>	34.83	23.36
<i>Anolis cristatellus</i>	23.23	41.27
<i>Anolis pulchellus</i>	13.54	
<i>Leptodactylus albilabris</i>	11.02	
<i>Anolis stratulus</i>		17.88
Average similarity (%)	30.18	48.55

The comparison among seasons showed that *Anolis cristatellus* and *Eleutherodactylus antillensis* characterized the two seasons in both areas with a contribution (%) greater than 10%. *Anolis cristatellus* in the west-wet obtained 32.22 % of abundance contribution. *Anolis pulchellus* characterized the east area both in dry and wet season. *Sphaerodactylus gaigae* characterized the west area during both seasons and

*Eleutherodactylus cochranae* characterized the west area only during the wet season. The highest average similarity percentage was in wet-dry, although the percentages obtained for the others seasons were higher than 55% representing difference between seasons (Table 4).

Table 4. SIMPER analysis of discriminating species in comparison among east and west areas and dry and wet seasons at the Vieques National Wildlife Refuge.

Species	Contribution (%)			
	East-Dry	East-Wet	West-Dry	West-Wet
<i>Anolis cristatellus</i>	29.16	26.76	28.46	32.22
<i>Anolis pulchellus</i>	23.94	23.27	9.49	
<i>Leptodactylus albilabris</i>	13.24	12.85		
<i>Sphaerodactylus macrolepis inigo</i>	12.37			
<i>Eleutherodactylus antillensis</i>	10.86	11.72	15.14	15.61
<i>Anolis stratulus</i>	6.27		18.17	9.44
<i>Sphaerodactylus gaigeae</i>			15.65	24.82
<i>Eleutherodactylus cochranae</i>				12.39
Average similarity (%)	71.41	80.81	81.17	68.42

A Multi-response Permutation Procedure (MRPP) and Indicator Species Analysis (ISA) was conducted to determine difference in composition community among wet and dry seasons and between west and east areas, and the results confirmed the previously found results.

With these tests a comparison of all east groups and sampling periods (AM/PM) with all west groups and sampling periods (AM/PM) was conducted to create only four groups separated by area and sampling period. The MRPP test indicated that the heterogeneity within groups was more than that expected by chance ( $A=0.1571$ ) with a significant difference ( $P<0.0000$ ). Pairwise comparisons indicated that there is a significant difference (all  $p<0.05$ ) in species composition between all study areas and sampling periods (Appendix B, Table 3). The result showed that even in study areas in

the same area, just by sampling at a different time the species composition changed. The ISA test found that eight out of eleven species were statistically significant in separating the different study areas with  $p < 0.05$  with relatively high importance values (Appendix B, Table 4). Out of all the species *Anolis pulchellus* was the species with the highest importance value (IV = 69.3%  $P = 0.0002$ ) and can be used to represent areas in the east sampled during the night. *Anolis stratulus* was another important species (IV=50.7) that can be used to represent areas in the west sampled during the morning.

The correlation values calculated in the west and east area for the relationship between richness species and abundance with the relatively humidity and precipitation were very low. Only the relationship with temperature in the west area showed significant statistical differences and the higher values in comparison with the east area (Table 5, Figure 12).

Species accumulation curves showed high representation of the documented herpetofaunal. The study documented a 100% of the expected amphibians and reptiles species in the west side (Figure 13 b,d). On the other hand, the study at the east area documented a 100% of the expected amphibian species, but for reptile species presented a 68.6 % and 63.2 % for the Incidence based coverage Estimator (ICE) and the Abundance –based Coverage Estimator (ACE) estimators respectively (Figure 13 a,c).

Table 5. Spearman correlation coefficients among the richness species and abundance at the west and east areas with the temperature, relative humidity and precipitation.

Correlation coefficients	Study area	Temperature (° C)	Rel. Humidity (%)	Precipitation (mm)
Richness species (S)	West	0.67866	- 0.25808	- 0.53835
	East	0.46146	0.065923	- 0.18743
Abundance	West	0.71982	- 0.56037	- 0.1742
	East	0.29091	- 0.1	0.044611

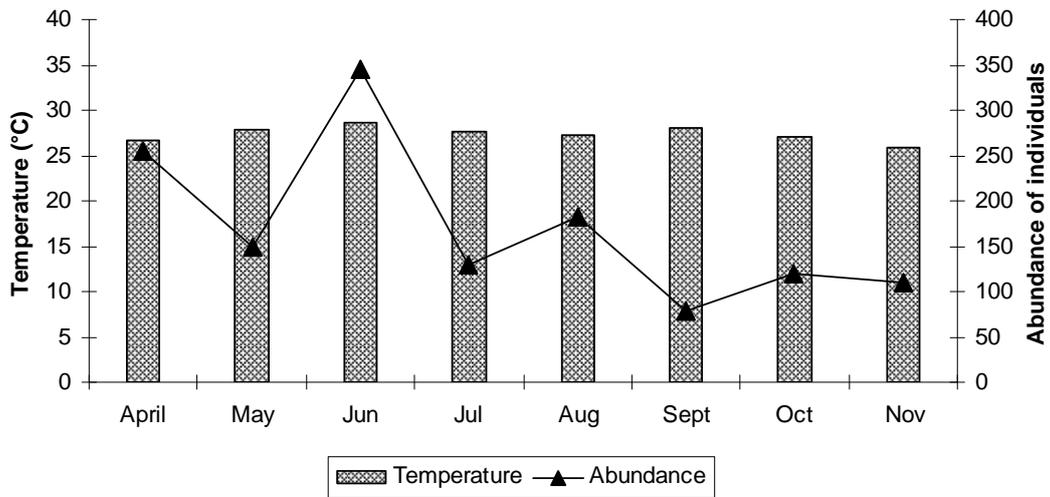


Figure 12. Monthly variation of the abundance of individuals in relation with the temperature levels during the sampling months at the west area of the Vieques National Wildlife Refuge.

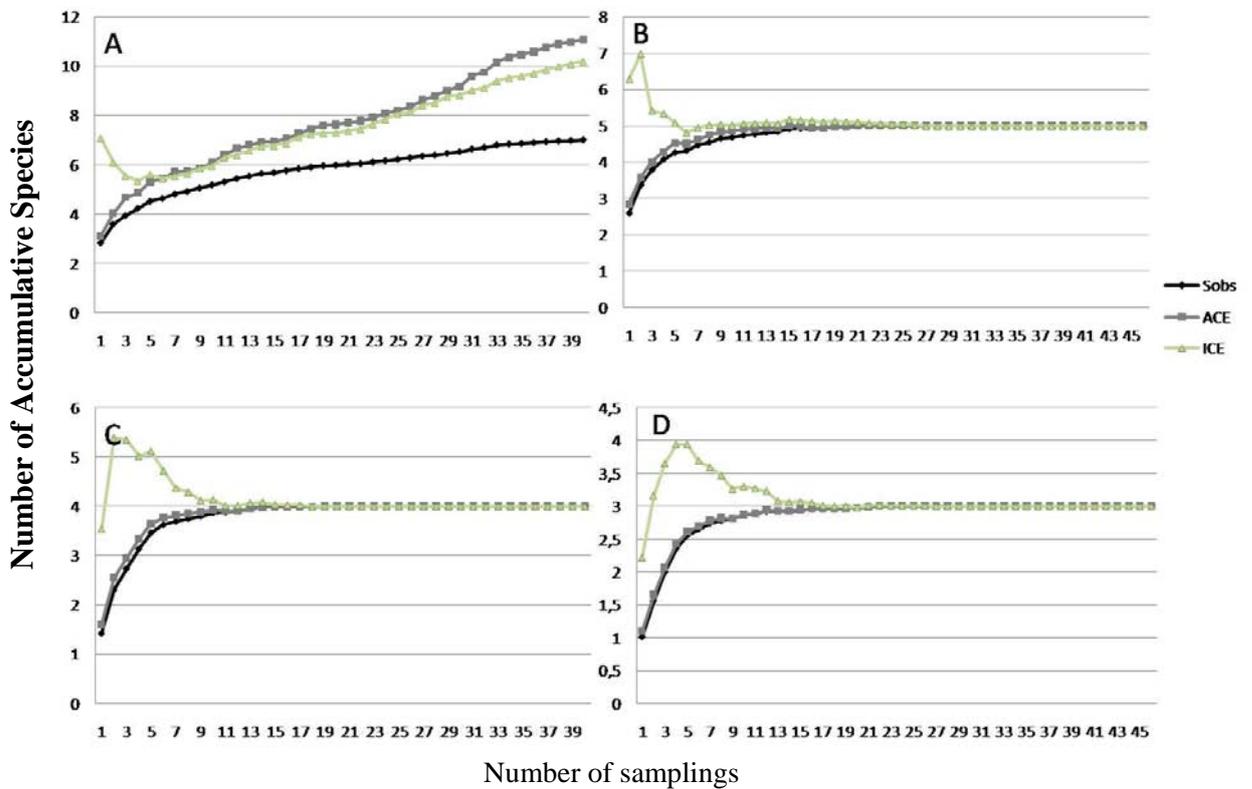


Figure 13. Species accumulation curves for reptiles (A, east; B, west) and amphibians (C, east; D, west) documented at the east and west areas of the Vieques National Wildlife Refuge.

The accumulation curve for reptile species in the east area shows that study sites have been highly sampled. Based on the models built to estimate the population size for *Anolis cristatellus*, the best model for both west and east areas was different capture and recapture probabilities with the captures time dependent and the recaptures dependent on environmental variables (Table 6, 7). The population size estimation for the east area was 255 individuals (SE: 0.0003; CI: 255 – 255.00003) and for the west area was 209 individuals (SE: 0.00008; CI: 209 – 209.00001), which indicates that the bigger population of *Anolis cristatellus* was found on the study sites at the east area of Vieques.

Table 6. Models for the population size estimation of *A. cristatellus* at the east area of the Vieques National Wildlife Refuge.

Model	AICc	AICc Weight
$P(t) \neq c(\text{envi})$	-326,326	0,99401
$P(t) \neq c(t)$	-316,102	0,00599
$P(\text{envi}) \neq c(\text{envi})$	-290,235	0,00000
$P(.) \neq c(\text{envi})$	-289,576	0,00000
$P(\text{envi}) \neq c(t)$	-280,114	0,00000
$P(\text{envi}) \neq c(.)$	-262,544	0,00000
$P(.) \neq c(.)$	-261,886	0,00000
$P(t) = c(t)$	-253,040	0,00000
$P(\text{envi}) = c(\text{envi})$	-217,804	0,00000
$P(.) = c(.)$	-211,798	0,00000
Mh	-209,793	0,00000

The recapture probabilities for the east area were higher in the dry season with a probability of 23.6 % (IC: 18.2 – 30 %) in comparison with the wet season with 8.9 % (IC: 7.3 – 10.9 %). On the west area the same pattern was obtained, but the recapture probabilities were lower than on the east area; the recapture probability during the dry season was 18.59 % (IC: 14.38 – 23.69 %) and during the wet season was 10.8 % (IC: 8.8 – 13.1 %).

Table 7. Models for the population size estimation of *A. cristatellus* at the west area of the Vieques National Wildlife Refuge.

Model	AICc	AICc Weight
$P(t) \neq c(\text{envi})$	-70,389	0,96355
$P(t) \neq c(t)$	-63,840	0,03645
$P(\text{envi}) \neq c(\text{envi})$	-18,376	0,00000
$P(.) \neq c(\text{envi})$	-13,539	0,00000
$P(\text{envi}) \neq c(t)$	-7,115	0,00000
$P(\text{envi}) \neq c(.)$	-5,189	0,00000
$P(.) \neq c(.)$	4,971	0,00000
$P(t) = c(t)$	13,320	0,00000
$P(\text{envi}) = c(\text{envi})$	34,287	0,00000
$P(.) = c(.)$	35,777	0,00000
Mh	36,292	0,00000

## DISCUSSION

The comprehensive conservation plan (CCP) and environmental impact statement for the VNWR (U.S Fish and Wildlife Service, 2007), the last document to list the herpetofaunal species on Vieques island, confirms the presence of 21 terrestrial herpetofaunal species including 1 toad, 4 frogs, 3 snakes, 12 lizards and 1 turtle species. Six more species, 5 snakes and 1 lizard, have been reported and may occur on the island for a total of 27 species, excluding the four sea turtles species which have been widely reported nesting and foraging along the coastal zones. The VNWR is the second largest area, occupying 17,771 acres administered by the federal or the commonwealth governments in Puerto Rico, following El Yunque National Forest, in Puerto Rico. According to information available, only 12 herpetofaunal studies have been conducted on Vieques; these focused on species presence. Eleven of the 27 potential terrestrial species (40.7%) were documented in my study (Appendix B, Table 5). The amphibian species documented were 4 out of 5 previously reported. Also a new exotic species (*Osteopilus septentrionalis*) was observed during the study. Of the reptile species expected to be found, 10 out of 16 (62.5%) previously reported were observed in areas within of VNRW, seven were documented throughout all study areas and three were occasionally encountered (*Typhlops hypomethes*, *Amphisbaena caeca* and *Ameiva exsul*). On the other hand, *Magliophis exiguus* was documented only at the east area of island, while *Anolis cuvieri*, *Borikenophis portoricensis aphantus* and *Typhlops platycephalus*, not were observed anywhere in the study areas.

The presence on the island of Puerto Rico giant Anole (*Anolis cuvieri*), the endangered species snakes Puerto Rican boa (*Epicrates inornatus*) and Virgin Island tree Boa (*Epicrates monensis granti*) is uncertain, although the habitat characteristics described by Rivero (1998) and Wunderle et al (2004) are present in several places in

Vieques. Only three individuals of Puerto Rican boa have been reported in the island and all these probably were pets illegally brought from Puerto Rico that escaped from their owners. No reports of Virgin Island tree boa has been documented, although the island has suitable coastal habitat for the presence of the species. No Puerto Rico giant Anole was observed in the study areas, although many of these contain potential habitats for the presence of this lizard especially in the mature secondary forest in the Monte Pirata area. Due to the low diversity and abundance of snake species documented in this study, I can suggest that the density of snakes is very low, and as result the encounters in the field are scarce. One possible factor that contributes to the absence of snakes on the island could be the introduction of invasive terrestrial mammals such as cats (*Felis catus*), rats (*Rattus sp.*) and Indian mongooses (*Herpestes auropunctatus*) by settlers. The Indian mongoose has been implicated in the extinction or extirpation of several species of *Alsophis* from major islands, including *Hypsirhynchus* (formerly *Alsophis*) *ater* from Jamaica, probably *Hypsirhynchus melanichnus* from Hispaniola, and *Borikenophis sanctaecrucis* from St. Croix, U.S. Virgin Islands (Henderson, 1992; Henderson and Sajdak, 1996).

The Antillean painted turtle (*Trachemys stejnegeri stejnegeri*) was not documented in the study areas, although individuals have been seen in urban areas and in other areas of the VNWR near intermittent creeks or in temporary fresh water ponds. The introduced red-eared slider (*Trachemys scripta*) could possibly be attributed to the pet trade.

The species richness values among the west and east areas were similar showing no statistical significant differences in diversity. From the 11 species documented in the entire study, 8 species were common in the west and east areas of the island. The distribution of species across the east and west area of the Vieques National Wildlife Refuge was not highly varied; however, there is reason to believe that certain species

were only found on particular sites due to the anthropogenic disturbances and lack of observations. The presence of *Hemidactylus mabouia* and *Magliophis exiguus* in the east area and not in the west area could be attributed to opportunistic observations, because only one individual per species was recorded during this study, that means that probability of finding these two species in the west area is also high. These two species have been widely reported in similar forests in Puerto Rico. The third species documented only in the east area was *Osteopilus septentrionalis*, the presence of this exotic and introduced species could be attributed to the U.S. Navy. Equipment and/or vessels and personnel from Guantanamo Naval Base (Cuba) were transported to this part of the island which was a training range for six decades.

### ***East area***

The eastern area of the Vieques National Wildlife Refuge was the region most disturbed during the Navy presence. Prior to the military uses, sugarcane production and livestock activities were established in these lands. From the mid-1940 until 2003 these land were used by the Navy and other military units for aerial bombing, artillery, small arms practice and infantry maneuvers (U.S Fish and Wildlife Service, 2007). Despite the disturbance level generated since 1940's by the military practices in the different ecosystems in the eastern part of Vieques, the herpetofaunal composition does not show impact in terms of species richness possibly due to the species present which are generalist species that due to its population dynamics have been adapted to disturbed environments with over the years in Vieques.

Grant (1932) reported the last herpetofaunal species list for Vieques before the presence of US Navy and Marine Corps. In that report he observed 11 species, two amphibians and nine land reptiles. In addition 4 species were reported but using the last

records taken from Schmidt (1928) and Reinhardt and Luetken (1862) for a total of 15 species before the presence of the US Navy and Marine Corps on 1940's. This study confirmed the presence of 10 of out 15 species reported before the 1940's and include one introduced species, *Osteopilus septentrionalis*. The absence in this study of the 5 remaining species could be due to the limited areas sampled (restricted access) established during the study, although the six sampling areas represented the 90% of all the types of habitats in the east area (excluding coastal and water bodies areas), the sampling area covered was 2,350 m<sup>2</sup> which represent less of 1% of total cover on the east area of Vieques. Approximately 10,000 acres of this area of the island have limited access due to the presence of unexploded ordnance (UXO's) in the soil and are in the process of being cleaned up by companies contracted by the US Navy. Perhaps the inability to sampling this area might be a reason which contributes to the fact that no *Sphaerodactylus roosevelti* were found, whereas Grant (1932) and Thomas (Personal communication) report this species in the island. Also, the unsuccessful drift fence arrays with pitfall and funnel traps could be other factor that reduced the possibility to detect the remaining species.

Despite the land uses and disturbances that have affected this part of the island; this study showed that the east area appears to have the higher herpetofaunal composition and abundance with eleven species registered in the 6 study sites within the Vieques National Wildlife Refuge. All the species documented throughout the Vieques National Wildlife Refuge were found in the east area of the island, although with lower abundance of *Eleutherodactylus antillensis*, *E. cochranæ* and *Sphaerodactylus gaigae* in comparison with the west area. This could be attributed to the subtropical moist forest present in the west area which provides suitable habitats for these amphibian and reptile species.

Of the 1,879 individuals registered, 1,860 (98.98%) were documented in three of five study sites where the visual encounters transects (VES) were established. VES was the most effective technique to measure the species composition and relative abundance in this study. Day and night surveillance of the transects covered the peak of activity for reptiles and amphibians found in the study sites.

The low number of individuals captured in drift fence arrays with pitfall traps was not expected. Only 19 individuals of 4 species were documented with this capture technique. One of the reasons could be the low diversity of species which are predominantly on the ground looking for food. In this study only one snake and two gecko species are restricted to the ground for perching or feeding on it. In previous field observations, geckos were observed inside the pitfall traps, but they could easily get out of the bucket using the lamellae in their feet. On the other hand, *Eleutherodactylus antillensis* was the amphibian most common in the pitfalls traps. One reason for these captures is due to the fact that the species is very common in the forest floor, where it feeds on ants, flies, beetles and leafhoppers (Henderson and Powell, 2009) and during daytime it hides under the leaf litter and loose bark of trees (Rivero, 1998). Snake species and other legless lizards were not registered in the pitfall traps or in the visual encounter transect implying that the snakes composition and abundance in the Vieques National Wildlife Refuge is low.

During the first 6 months of this study the material used for the drift fence was silt fence. Due to the low capture success during the last 2 months the material was changed to aluminum and galvanized steel to try to decrease the climbing ability of anoles using their subdigital toepads and claws but there was not difference in the number of individuals captured after the change of drift fence material. The higher abundance observed in the dry season in comparison with wet season could be due to fact that less

amount of sampling were conducted during the wet season due to the heavy rains which did not allow access to study areas. However, there is no influence on the abundance. *Anolis cristatellus* was the most abundant species during the dry and wet seasons, due to its adaptations to deal with altered habitat (Powell and Henderson, 2008) and prolonged wet and dry periods (Perry, 2005). The distribution of species across the six study sites in the east area was not highly varied due to the similarity of habitat among transects. Amphibian species *Eleutherodactylus antillensis* and *Osteopilus septentrionalis* showed a high abundance during the wet season. They were observed to be very active during the night surveys in tree branches. Based in the SIMPER analysis *Anolis cristatellus* and *Eleutherodactylus antillensis* were the species that provided the higher percentage contributions in terms of total abundance among areas and seasons indicating that both species were present at the areas and seasons. *Anolis pulchellus* was the second most abundant reptile species with high abundance in both seasons and higher abundance in lands dominated by grass and shrubs in three of six sites at the east area. The changes in species richness observed throughout the study were not significant. The absence and low abundance of 2 of 3 species could be explained by the secretive habits, and foraging places that were difficult to detect, and in some degree the sampling techniques used. *Sphaerodactylus gaigae* has a preference for moist habitat found in the west area of Vieques, where the abundance of leaf litter provides more food resources, shelter places as well as restricted microhabitat within the leaf-litter. Similar habitat conditions were found in Lopez-Ortiz and Lewis (2004) with *Sphaerodactylus nicholsi* in the southwestern part of Puerto Rico where the habitat selection was characterized by patches with protection from direct sunlight by evergreen canopies, thick leaf litter and trees that contribute large seeds or dry fruit to the litter. The combination of these interrelated features, make a suitable microhabitat where this species can live. Although 9 individuals

of *Sphaerodactylus gaigae* were found on the east area, they occupied shady places and leaf litter where the percentage of canopy cover is greater than 60%. The herpetofaunal composition on the east area of Vieques is dominated by seven species of land reptiles which provided the 80.15% of all herpetofaunal species documented. The reptile community is mainly represented by Anoles lizards distributed in different ecomorphs over three of six study sites in the east area. Ecomorphs refers to different structural microhabitats (Williams, 1972) and other characteristics as head dimensions (Harmon et al., 2005) and limb muscle mass (Vanhooydonck et al., 2006). The three Anole species documented in this study occupy different ecomorphs, trunk-ground (*Anolis cristatellus*); trunk-crown (*Anolis stratulus*) and grass-bush (*Anolis pulchellus*).

Although higher species richness was provided on the east area, the diversity indices calculated for the amphibians and reptiles were low for the east and west areas. Based on the results of Simpson index the dominance also was relatively low for both area. According to Marrugan (2004) increased dominance results in decreased diversity, for this reason the low diversity is not influenced by dominance of a few species such as *Anolis cristatellus* or *Anolis pulchellus*. This can be attributed to the few herpetofaunal species present and documented in the island which represents only 13.58% of the all herpetofaunal species reported for Puerto Rico Island including satellite islands.

Population size calculated for *Anolis cristatellus* based on the capture - recapture method could represent reliable estimates of population size for this lizard in the study areas, since it was the most abundant species found over the study and can tolerate extremely dry habitats presenting a low cutaneous water loss rates and high skin resistance to water vapour (Dmil'el et al 1977; Perry et al. 1999).

Revell (personal communication, 2010) captured about 600 adult males in approximately 2 hectares in Cayo de Tierra (south west of Vieques); this means that there

would be at least 1,200 adult *Anolis cristatellus* if given equal sex ratio. In unpublished reports with the Desecheo Anole (*Anolis desecheensis*), a species with a general ecology and structural habitat very similar to *Anolis cristatellus*, 125 individuals were observed in 0.237 hectares; these population size are in the range of the estimate calculated for the east area where the sampling areas present a relatively high abundance.

The recapture probabilities are greater during the dry than the wet season, because the lizard's detectability is higher during the time periods without rain when it can conduct for long periods primary activities like thermoregulation and perching in the structural habitat.

The accumulation curves suggest that sampling efforts in the east area were 100% efficient to characterize the amphibian species composition and 68.6% for the reptile species composition. The seven land reptiles species documented in this study represent more than 40% of all land reptiles species reported throughout the island. Ten reptiles species were not documented in this study, *Borikenophis* (formerly *Alsophis*) *portoricensis aphantus* (possibly extinct, Rodriguez-Robles, 2005); *Typhlops hypomethes* (Rivero, 1998; two individuals were found in the west side and several sightings in house gardens in the urban areas); *Typhlops platycephalus* (Rivero, 1998); *Typhlops richardi* (U.S Fish and Wildlife Service, 2007). *Amphisbaena caeca* (Rivero, 1998; two individuals were found on the west area in opportunistic encounters out of the study sites); *Iguana iguana* (several individuals observed out of the study sites, especially in coastal margins); *Sphaerodactylus roosevelti* (Grant, 1932; documented in the extreme eastern dry tip of the Island); *Mabuya sloanii* (Rivero, 1998); *Ameiva exsul* (Rivero, 1998, several individuals was observed in Cayo de Tierra, out of the study sites); and *Trachemys stejnegeri stejnegeri* (Rivero, 1998, several individuals observed in

intermittent ponds and creeks in urban areas or other areas of the VNWR, but out of the study sites).

According to the literature, most of these rare species are captured using drift fence arrays with pitfall traps (Heyer et al, 1994). In this study the probably that the abundance of certain species is very low increased the difficulty of detecting these secretive and uncommon species in the dry forest.

### ***West area***

The western portion of the Vieques National Wildlife Refuge covers approximately 3,100 acres which represent 9.35% of the total surface of Vieques Island. The use and management of these lands since 1940 until 2001 by the U.S. Navy has had less anthropogenic impact compared with the eastern portion of the island. Military constructions and activities such as munitions storage in bunkers, a communication facility at Monte Pirata, an open burn/open disposal site near Punta Boca Quebrada, and recreational sites at Punta Arenas were established within these lands (CCP and environmental impact statement, 2007). Before the U.S. Navy presence, most of these lands were used extensively for timber extraction and agricultural activities such as sugar cane plantations. Some forested lands with higher elevations such as Monte Pirata hillsides, where a mature secondary forest which covers approximately 29.2% of the area, were not affected directly by agriculture. Although the level of disturbance in the types of vegetation that cover the VNWR throughout time has been modest, only 8 of the 27 herpetofaunal species (29%) previously reported for the Vieques island were documented in the five study areas in the western area; three less compared with the eastern area, but only one amphibian species (*Osteopilus septentrionalis*) was absent on the western area. Four of five sampling areas were established in high elevations in the Monte Pirata

hillsides and places near to intermittent creeks, where species abundance and richness of amphibians could be larger (Gould et al 2008) than found on the eastern area and could potentially exceed the richness of reptiles. However, the higher species abundance and richness were provided by the reptiles, with 1,037 individuals of 5 species whereas the amphibians provided 332 individuals of 3 species. *Anolis cristatellus* and *Sphaerodactylus gaigeae* were the reptile species most abundant in the western side followed by *Anolis stratulus*. The abundance of *Anolis cristatellus* is lower compared to the east area. This difference could be attributed to the reduced detectability in closed forest where the basking sites are few and a high tolerance to variable temperatures (Huey, 1983). *Sphaerodactylus gaigeae* was the second most abundant reptile species, particularly under the leaf litter, rocks and logs in the hillsides of Monte Pirata.

*Eleutherodactylus antillensis* was the most common amphibian species found in the west area with a wide distribution in all vegetations types, showing a high abundance in the mature secondary forest associated with hillsides, hilltops and closed canopy forest. The distribution and microhabitat selection observed for this species in Vieques, coincides with that reported by Stejneger (1904), Rivero (1998); and Stewart and Woolbright (1996). The higher abundance on the western area in comparison with the eastern area is due to forest type which receives more rainfall during the year. Also provides low temperatures, moist vegetation and food such as ants, flies and beetles (Jones, 1982).

*Eleutherodactylus cochranae* was the second most abundant amphibian species, although it also was found in the dry forest (east area), the abundance in the moist forest was higher due to the axils of arboreal bromeliads (*Tillandsia*), mature trees and the cavities in latter, provide retreats during the day. During the night were very common on leaves and tree trunks. Similar to the eastern area, the visual encounter transects were the most efficient survey technique to document species composition during this study.

Crosswhite et al (1999) also found that the visual searches were more effective in detecting herpetofaunal species compared to drift fence arrays with pitfall traps, although the visual encounter surveys are more labor intensive during the sampling period. Over 95% of the individuals were encountered during the daily and nightly transects by visual searches and only 2.19% were documented in the pitfall and funnel traps. Drift fence with pitfall and funnel traps was placed on the hillsides of Monte Pirata in locations with slopes.

On the east area the drift fence was established on level ground, but there was no difference in effectiveness between east and west. Thirty individuals of 6 species were encountered in the pitfall traps, with *Anolis cristatellus* and *Eleutherodactylus antillensis* being the species with more individuals captured, due to their microhabitat preferences and perhaps a reduced ability to escape the pitfall trap. No rare species such as snakes, blindsnakes or legless lizards were documented in pitfall or funnel traps although in other studies as Jones (1986) and Campbell and Christman (1982) it was an effective technique. Only individuals of the blindsnake (*Typhlops hypomethes*) and the legless lizard (*Amphisbaena caeca*) were observed in opportunistic encounters in areas away from the study sites. These rare species were not observed in the transects via visual searches either, so this suggests that the abundance of these reptiles species in the west area study sites is as low as on the east area.

The difference in abundance among dry and wet season, showed a seasonal pattern in the community structure, indicating that the abundance of certain herpetofaunal species is influenced by rainfall. *Eleutherodactylus cochranae* and *Anolis cristatellus* showed highest abundance in June at the end of the dry season and the beginning of the wet season. This decrease in abundance for *Anolis cristatellus* could be attributed to thermoregulation behavior. In the dry season with apparently more basking sites the

species is an effective thermoregulator, and during the rainy season it becomes a thermoconformer, thermoregulating more effectively in certain areas within the closed canopy forest, those its detectability decreases varies throughout the seasons (Hertz et al 1993).

The SIMPER analysis results demonstrated that *Anolis cristatellus* and *Sphaerodactylus gaigeae* were the species that provided the higher contributions in terms of total abundance among areas and seasons, also indicating that both species were present at the areas and seasons throughout the study. *Anolis stratulus* was the third most abundant reptile species present in both seasons, apparently being more abundant in the dry than in the wet season. This coincides with Reagan (1986, 1992) who conducted population studies at El Verde. The greater abundance of *Sphaerodactylus gaigeae* and *Anolis stratulus* in the west than in the east was probably due to the availability of microhabitats that the forested areas in the west region can provide such as dense and deep leaf litter layer, larger trees, shaded places, trunks on which camouflage can be most effective in the case of Anoles lizards. *Anolis stratulus* is trunk-crown ecomorph those preferring larger trees. The changes in richness throughout time were not significant, certain species such as *Anolis pulchellus* and *Sphaerodactylus macrolepis inigo* were absent in some sampling months generally during the wet season. Based on the results, the herpetofaunal composition in the west area is dominated by three reptile and two amphibian species which provided a 94.3% and 83.9% respectively of each herpetofaunal group.

The ecological index values reflect the same patterns observed in community structure. There was not a spatial pattern among study areas; in this case the dominance and evenness among areas were very similar. The diversity index was low for reptiles and amphibians, if we compared the number of species with the number of individuals

encountered, demonstrating the presence of few species represented by numerous individuals.

The population size calculated for *Anolis cristatellus* in the study areas showed that the west area has a lower population size than the east area with differences in the recapture probabilities among dry and wet season. One possible explanation for the difference in recapture probabilities increase in the dry season is because the sampling periods started in the months of dry season when the lizards are very active and exposed in basking sites during the day. This makes them easier to capture and subsequently recapture in the thermoregulation sites, as they usually exhibit site fidelity.

The accumulation curves for the reptiles and amphibians registered in the west area showed an asymptotic behaviour with a high representation of the amphibians and reptiles in the west area. The sampling efforts for the west area were efficient in 100% to characterize the herpetofaunal composition in the study areas with the survey techniques used, principally searches via visual encounter transects. The curves for amphibians and reptiles began to stabilize from the second month of sampling. The calculated estimators demonstrated that the study areas have been adequately and extensively sampled documenting all herpetofaunal species expected.

According to Brandeis et al (2007) the forests in Culebra and Vieques differ markedly from those on mainland Puerto Rico. Vieques's forest cover is now 85% and in average a hectare of subtropical moist forest on Vieques has 20 percent fewer trees than has a hectare of such forest on mainland Puerto Rico. With the sudden end of military exercises by the US Navy in the island the forest cover will have important changes positively affecting the existing fauna and flora.

The herpetofaunal community in Monte Pirata area which is one of the most conserved places of the island has had a similar species composition over the last forty years with

the exception of three species which were not documented in the study areas, but were encountered in other places of the island. The military activities in this portion of the island had not the same disturbance level as in the eastern half of the island. However, the species composition in the east area did not show expected impact from the military activities. Perhaps military activities may have allowed regrowth of forest and so improved conditions compare to the agricultural past of the region. Possibly the presence of introduced and invasive mammals species in the forested lands over all the Vieques island can be one of the main reasons of the low abundance and possible absence in the wild of semi-fossorial species such as *Magliophis exiguus*, terrestrial diurnal snakes (*Borikenophis portoricensis*), the species of *Epicrates*, which are crepuscular and nocturnal and generally arboreal snakes (Tolson and Henderson, 1993). Normally researchers do not encounter this highly efficient predator unless it is basking on the ground or hiding in an easily accessible refuge (Tolson and Henderson, 2006). Monte Pirata area presents a suitable habitat for the presence and possible abundance of snakes species due to the existing conditions of the mature secondary forest, but the absence of snakes species during the sampling period may correspond with the high abundance of invasive species such as mongooses (*Herpestes javanicus*) and black rats (*Rattus rattus*) observed during the daily and nightly surveys respectively. Control or eradication of invasive species as black and Norway rats, mongoose and cats in islands such as Vieques, could result in a significant increase in wildlife populations including birds and herpetofaunal species.

## **FUTURE WORK AND RECOMMENDATIONS**

This work was focused on the evaluation of the land herpetofaunal composition that occurred in eleven sites, which represent most of the ecosystems found within the Vieques National Wildlife Refuge. The results of this study demonstrate that the herpetofaunal species' richness among the western and eastern side of the Vieques National Wildlife Refuge are very similar and that the abundance of certain species is relatively different particularly among the dry and wet season. Three capture techniques were used to sample all the species present in the community and only one, the visual encounter survey transect, was effective to document the species richness and abundance of the eleven species documented in this study. Based on the results from this study, I would like to make the following recommendations:

1. Increase the study sites in order to develop long term monitoring programs for herpetofauna within the VNWR. This study was conducted during eight months covering both in dry and wet seasons. I recommend the establishment of a multiyear and long-term inventory and monitoring program as new areas become accessible within VNWR. This is an ongoing effort, as these areas are being cleared of UXO's. This will provide the establishment of baseline guides of population dynamics and community structure of the herpetofauna.

2. Due to the low detection of snake species' in all the study sites, using the three capture/detection techniques, I recommend the use of artificial cover-boards in places that have potential habitat for the presence of these rare species in the VNWR. This method has been used in many ecological studies. The detection/capture rate with this technique

can be low during the first months of monitoring, because animals might need time to locate the artificial refuges.

3. Because this study was conducted in areas not near the coast, I recommend the establishment of transects on the coastline in order to assess coastal habitat which might provide both habitat and refuge to endangered species such as the Virgin Island tree boa (*Epicrates monensis granti*).

4. Based in the field observations from this work, the high abundance of mammal invasive species such as mongoose and black rats throughout the VNWR could represent a factor contributing for the low abundance of snake species. The establishment of an aggressive eradication or control program for these invasive species is imperative to help in the recovery of the snake populations and maybe other herpetofaunal species within the VNWR.

5. Due to the historical changes in the land use in Vieques, including the VNWR, reforestation actions with native plant species, are recommended across the coastal and upland areas in the VNWR. Forest recovery, especially in the east side of the island, will provide more habitats for wildlife species, such as birds, amphibian and reptiles among others, in order to increase species' richness and abundance in populations which nowadays exhibits low counts of individuals due to unsuitable habitat conditions in some places within the VNWR.

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# APPENDICES

## APPENDIX A

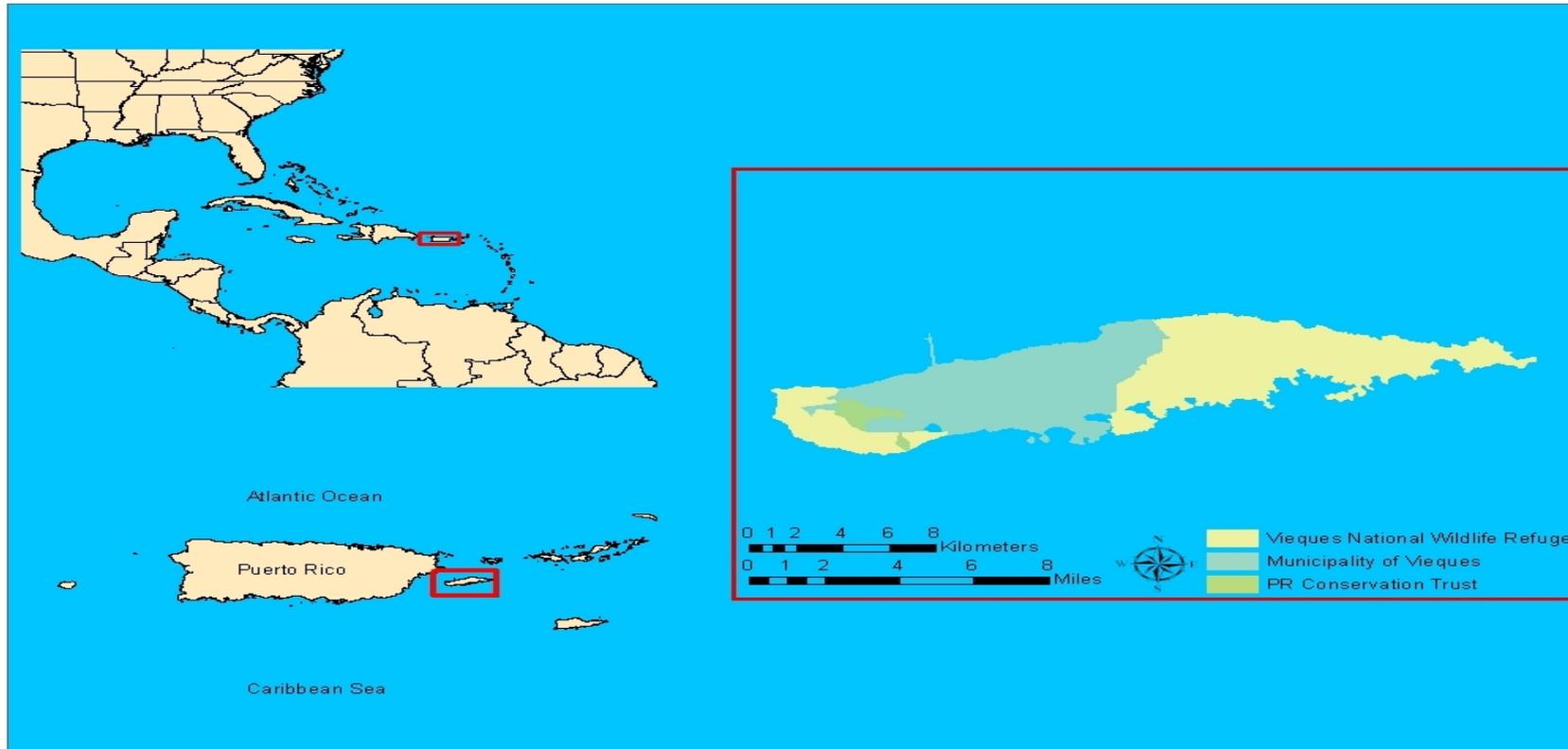


Figure 1. Island municipality of Vieques location map. Taken and modified with permission from Comprehensive Conservation Plan and Environmental Impact Statement, Vieques, 2007

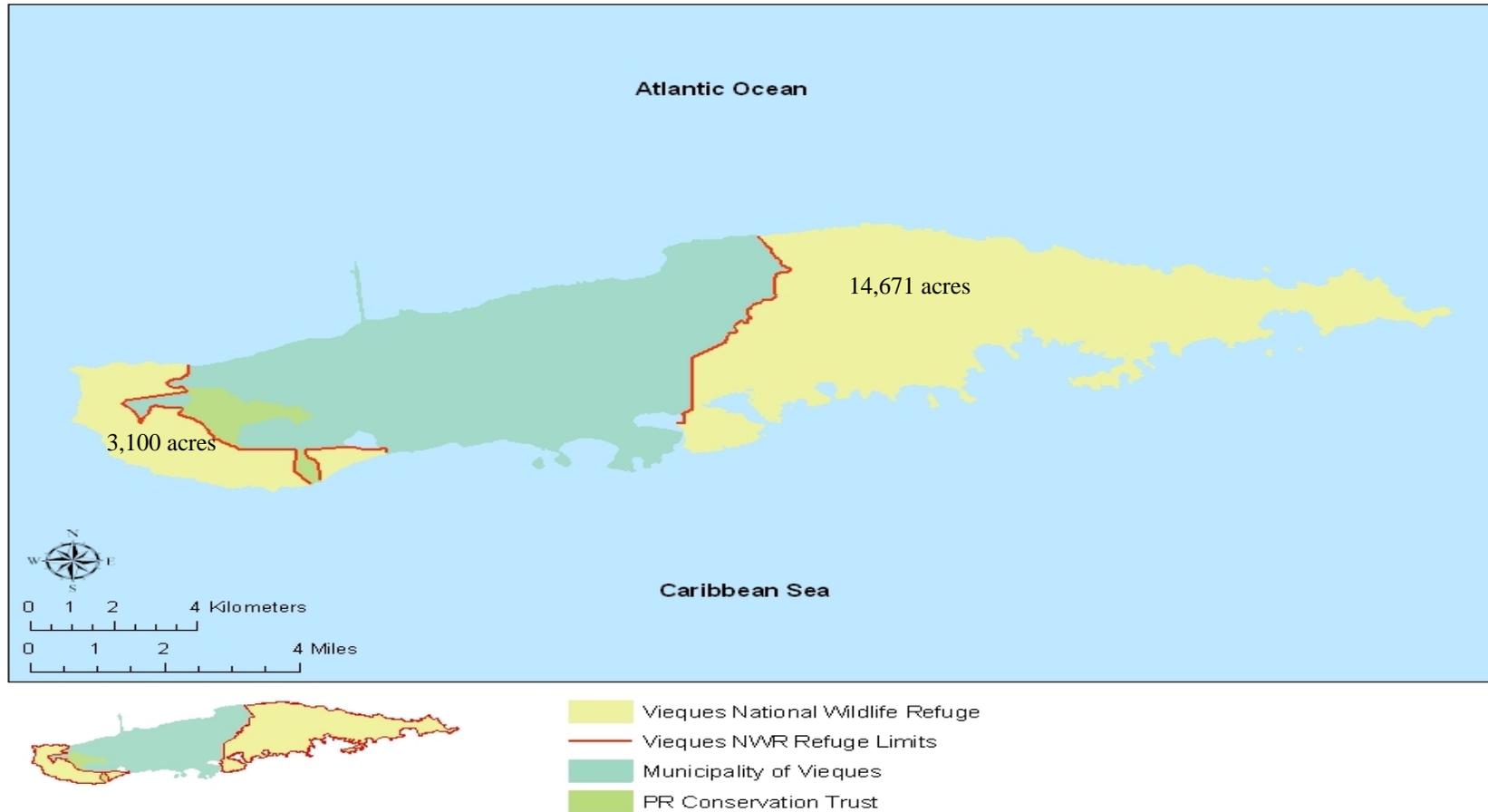


Figure 2. Surface area of the Vieques National Wildlife Refuge and their boundaries with the lands property Puerto Rico Conservation Trust and the Municipality of Vieques. Taken and modified with permission from Comprehensive Conservation Plan and Environmental Impact Statement, Vieques, 2007

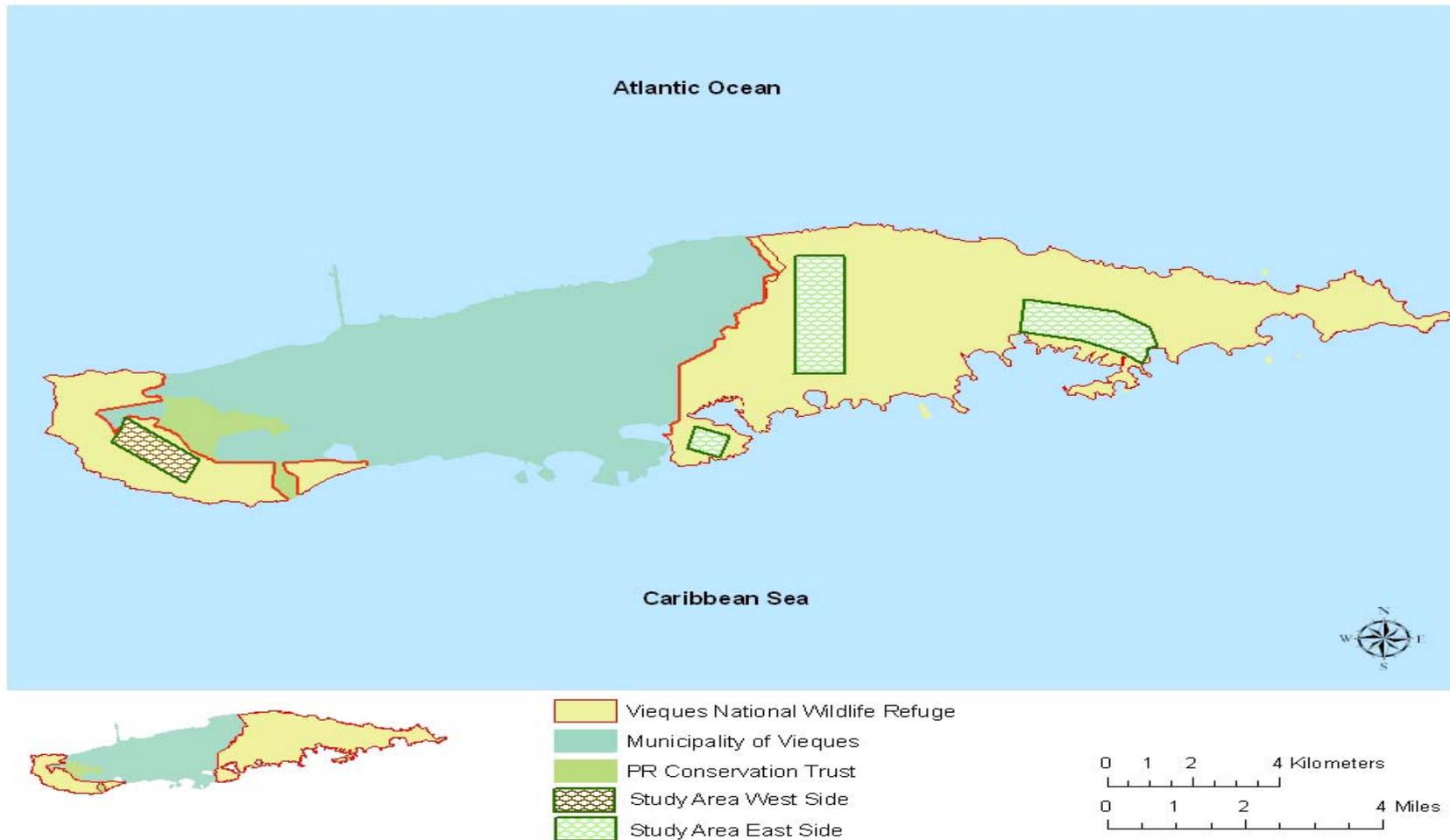
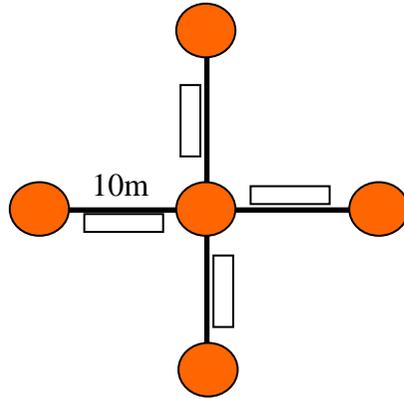


Figure 3. Location of the study sites at the east and west areas of the Vieques National Wildlife Refuge. Taken and modified with permission from Comprehensive Conservation Plan and Environmental Impact Statement, Vieques, 2007



4a



4b



4c

Figures 4a, 4b and 4c. Design for arrays of the drift fence composed for five pitfall buckets places at the end of the arms and in the center, and four double ended funnel traps. ——— Drift fence array; ● Pitfall trap; □ Double ended funnel traps.



**5a**



**5b**

Figures 5a and 5b. Leaf litter at the bottom of the pitfall bucket and lids of the bucket providing shade inside the bucket.



**6a**



**6b**

Figures 6a and 6b. Double ended funnel traps located in the arms of the drift fence arrays.

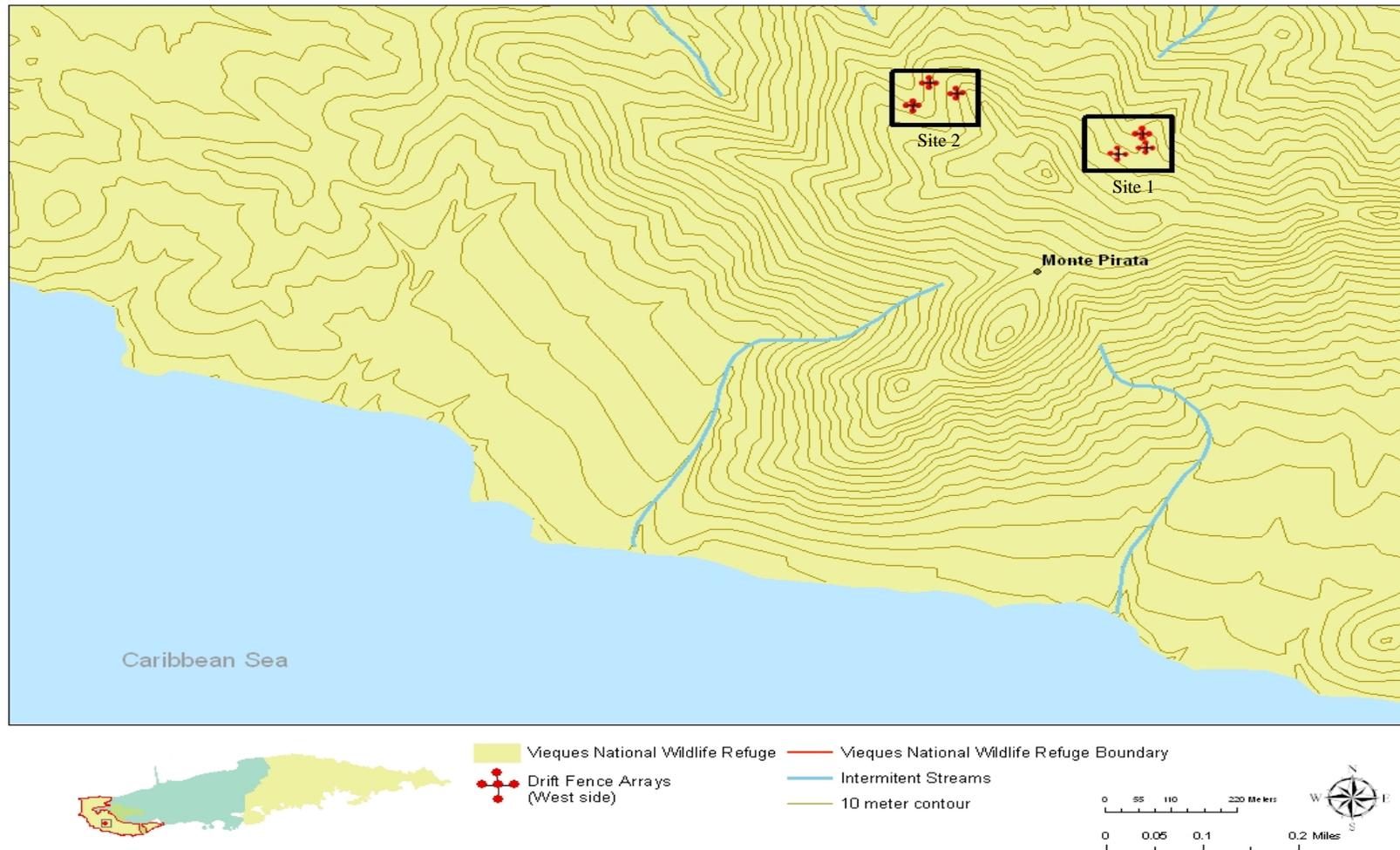


Figure 7. Locations of the six drift fence arrays in Monte Pirata area at west area of the Vieques Island.

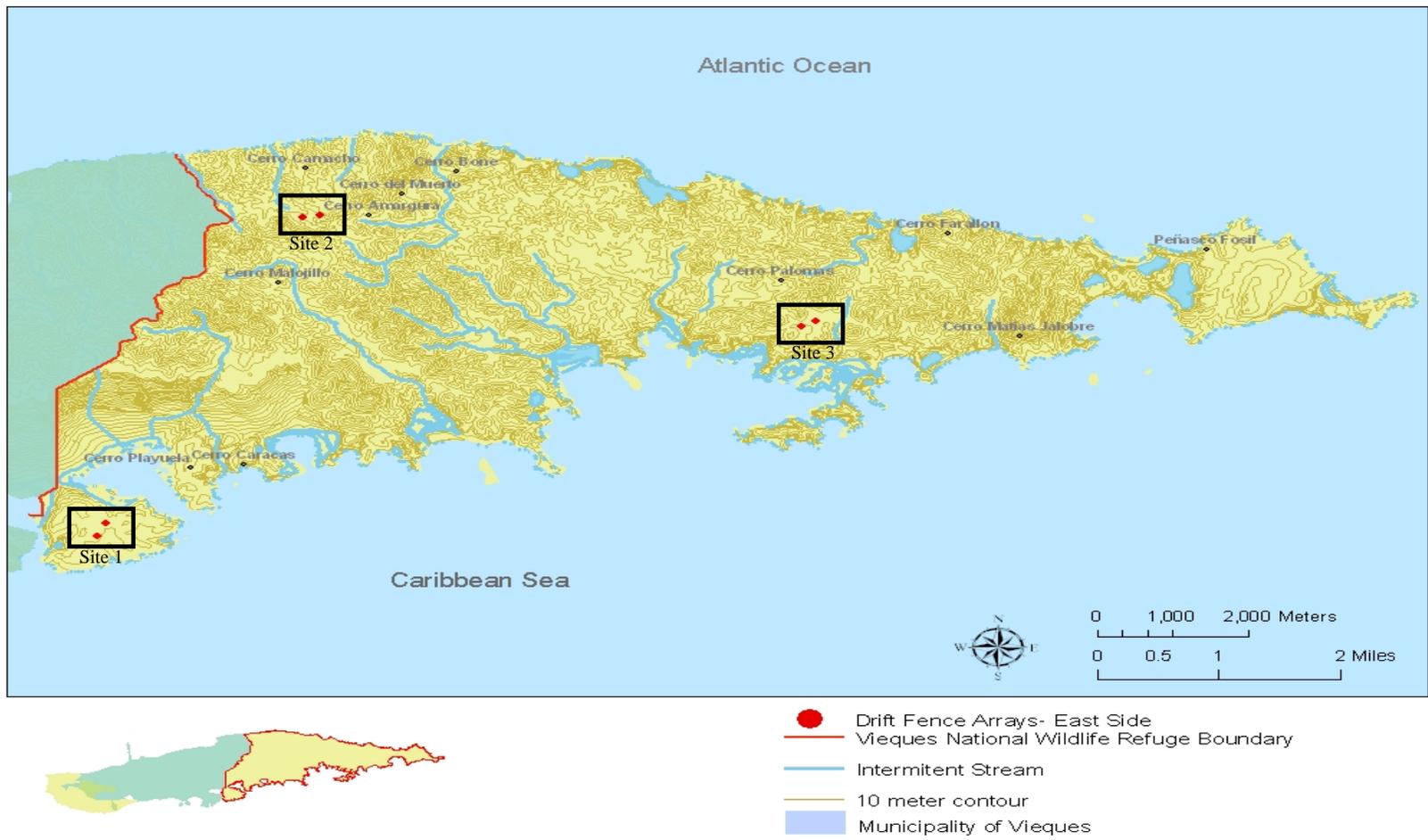


Figure 8. Locations of the six drift fence arrays at the east area of the Vieques Island. Taken and modified with permission from Comprehensive Conservation Plan and Environmental Impact Statement, Vieques, 2007

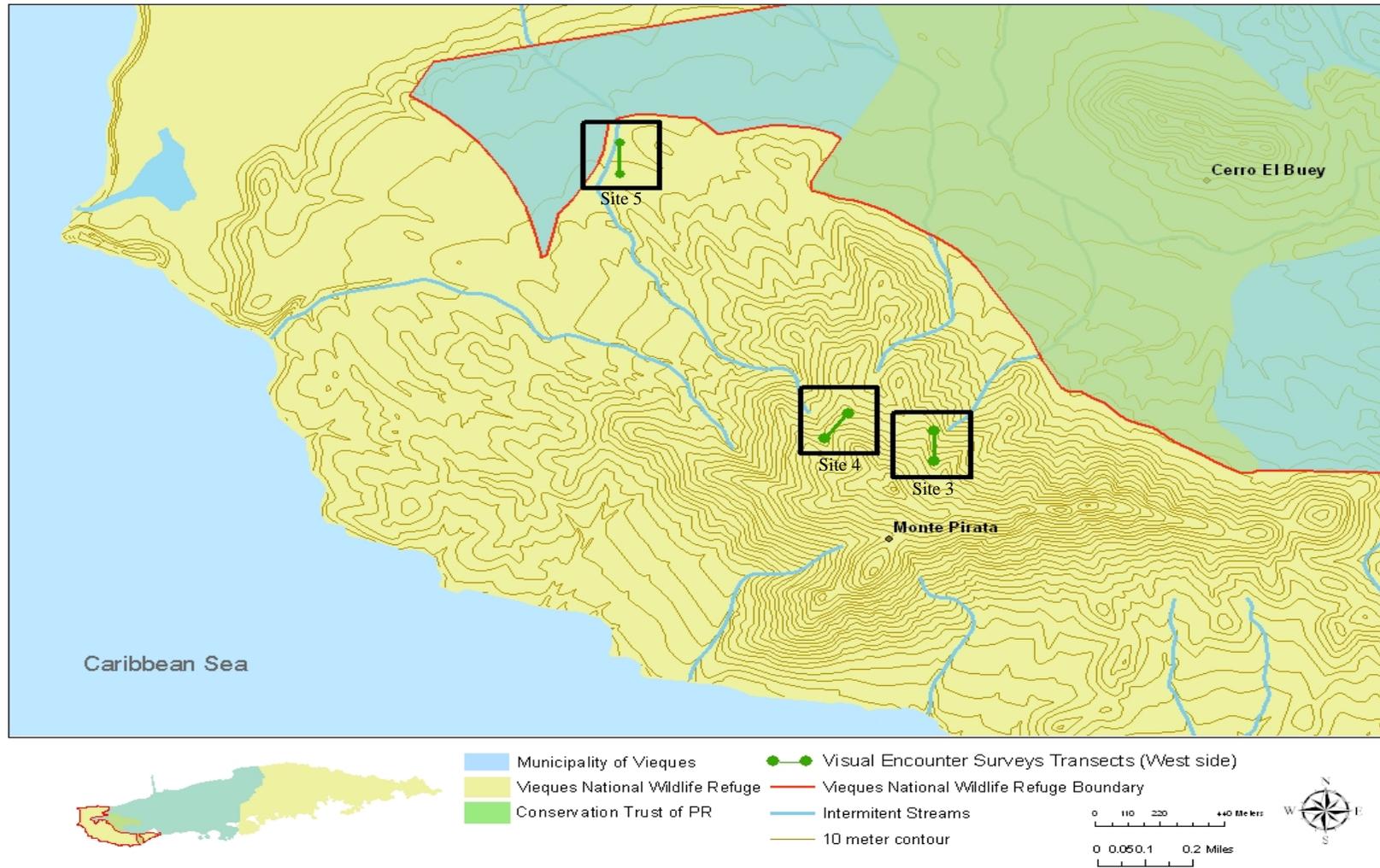


Figure 9. Locations of the three Visual Encounter Survey (VES) transects in Monte Pirata area at the west area of Vieques Island. Taken and modified with permission from Comprehensive Conservation Plan and Environmental Impact Statement, Vieques, 2007

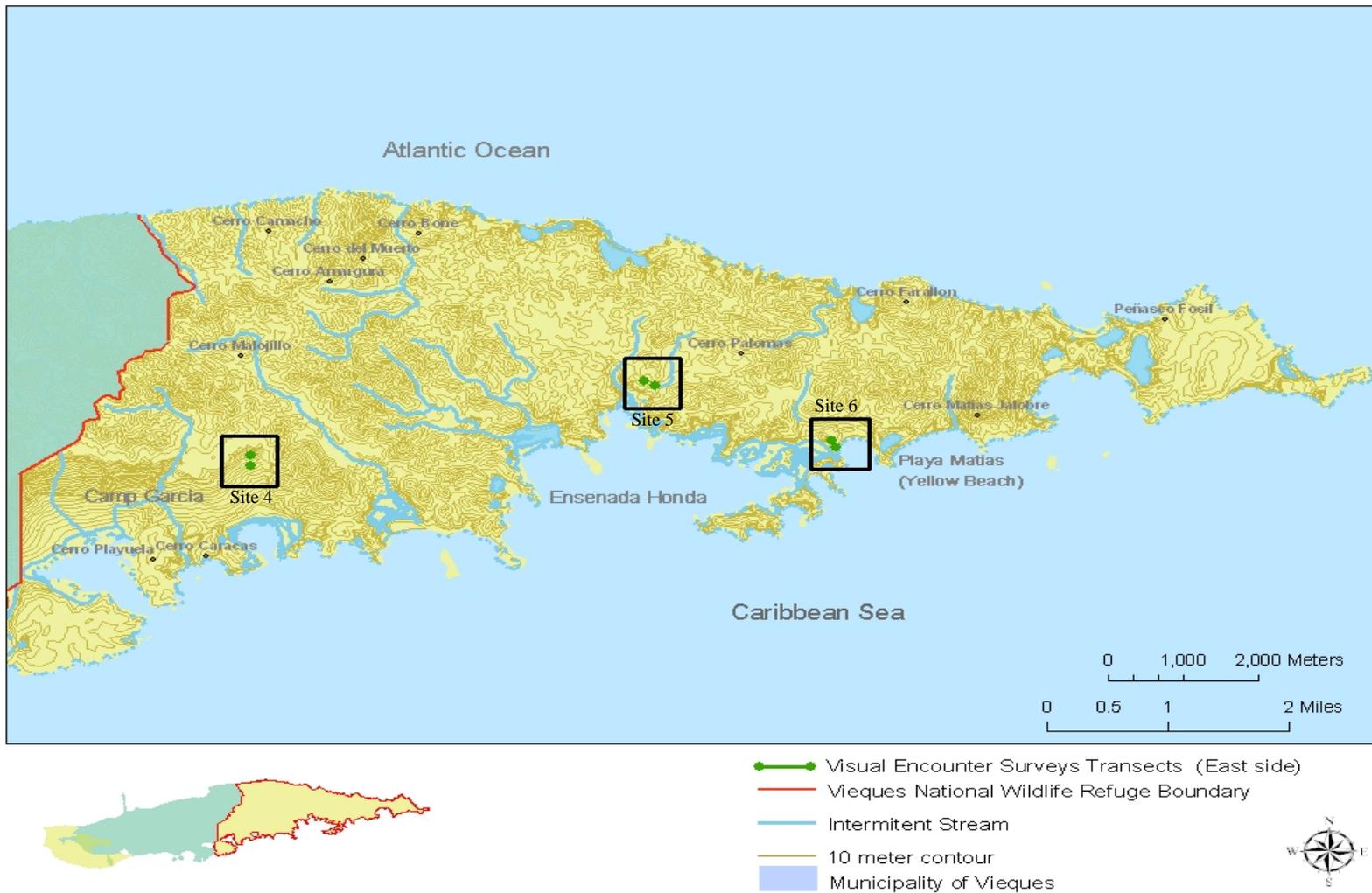


Figure 10. Locations of the three Visual Encounter Survey (VES) transects in the east area of Vieques Island. Taken and modified with permission from Comprehensive Conservation Plan and Environmental Impact Statement, Vieques, 2007

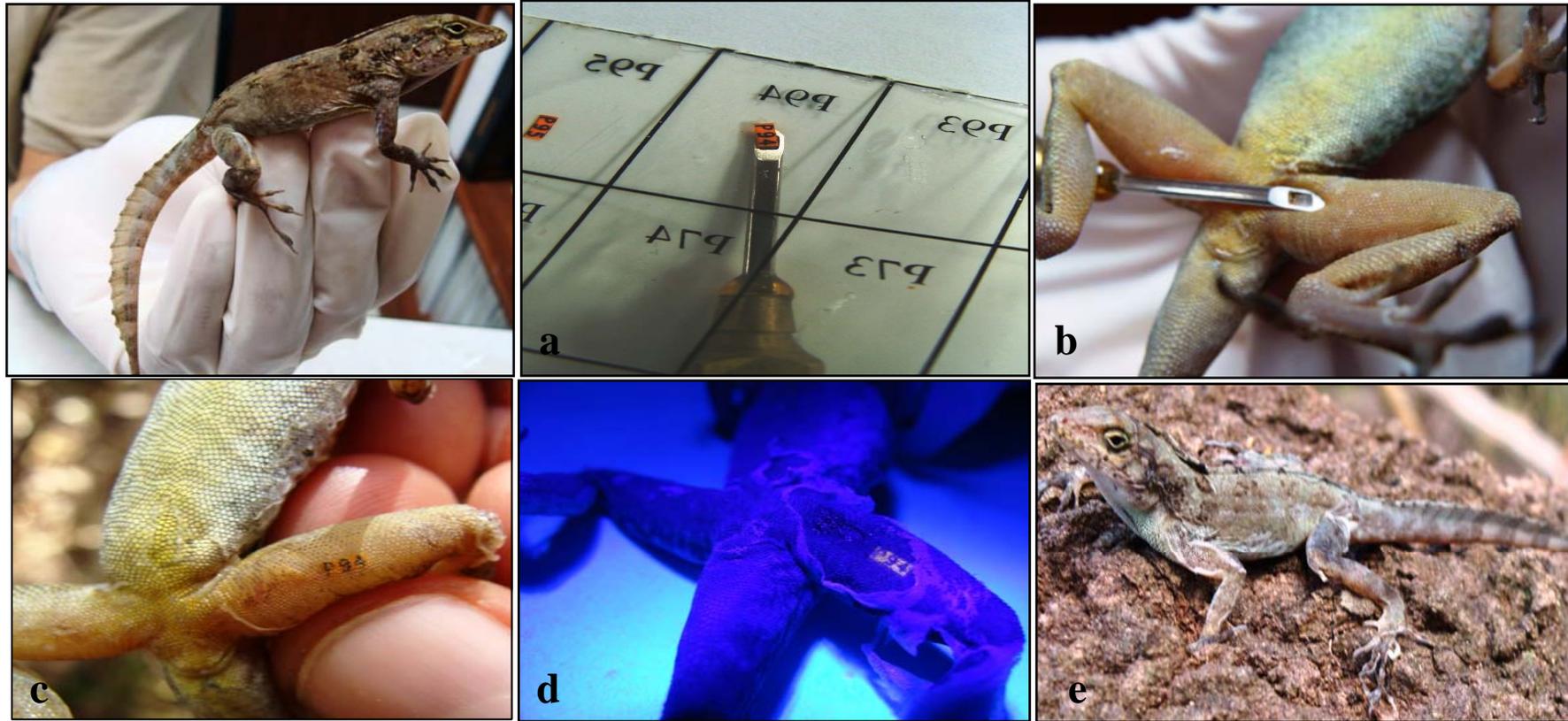


Figure 11. Implanting the Soft VI Alpha Tags. Once the lizard is captured, the first step is loading the tag into the injector (a), injecting the tag into the lizard (b), the lizard with the tag under the subcutaneous layer of the ventral side of a hind limb (c), reading the tag under an ultraviolet light (d), lizard released (e).

## APPENDIX B

Table 1. List of amphibian and reptile species captured and observed via drift fence arrays with pitfall traps and visual encounters surveys transects encountered in the study areas at the east area of the Vieques National Wildlife Refuge.

Family	Scientific name	Dry season						Wet season					
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Eleutherodactylidae	<i>Eleutherodactylus antillensis</i>	4	5	3	23	15	0	0	1	0	14	71	20
Eleutherodactylidae	<i>Eleutherodactylus cochranae</i>	0	0	0	0	20	0	0	0	0	0	14	2
Leptodactylidae	<i>Leptodactylus albilabris</i>	0	0	0	23	19	0	1	0	1	2	58	27
Hylidae	<i>Osteopilus septentrionalis</i>	0	0	0	4	10	2	0	0	0	0	12	22
Gekkonidae	<i>Hemidactylus mabouia</i>	0	0	0	1	0	0	0	0	0	0	0	0
Polychrotidae	<i>Anolis cristatellus</i>	0	0	2	138	109	203	1	0	0	97	181	180
Polychrotidae	<i>Anolis pulchellus</i>	0	0	0	60	97	57	0	0	0	29	142	82
Polychrotidae	<i>Anolis stratulus</i>	0	0	0	15	13	1	0	0	0	4	23	0
Sphaerodactylidae	<i>Sphaerodactylus gaigae</i>	0	0	0	0	1	1	0	0	0	0	6	0
Sphaerodactylidae	<i>Sphaerodactylus m. inigo</i>	0	1	0	6	9	16	0	0	0	6	11	13
Dipsadidae	<i>Magliophis exiguus</i>	0	0	0	1	0	0	0	0	0	0	0	0
<b>Total individuals</b>		<b>4</b>	<b>6</b>	<b>5</b>	<b>271</b>	<b>293</b>	<b>280</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>152</b>	<b>518</b>	<b>346</b>
<b>Total species</b>		<b>1</b>	<b>2</b>	<b>2</b>	<b>8</b>	<b>9</b>	<b>6</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>6</b>	<b>10</b>	<b>7</b>

Table 2. List of amphibians and reptiles species captured and observed via drift fence arrays with pitfall traps and visual encounters surveys transects encountered in the study areas at the west area of the Vieques National Wildlife Refuge.

Family	Species name	Dry season					Wet season				
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5
Eleutherodactylidae	<i>Eleutherodactylus antillensis</i>	3	2	31	40	16	2	2	44	38	0
Eleutherodactylidae	<i>Eleutherodactylus cochranæ</i>	0	0	24	27	4	1	0	27	18	0
Leptodactylidae	<i>Leptodactylus albilabris</i>	0	0	23	6	5	0	1	18	0	0
Hylidae	<i>Osteopilus septentrionalis</i>	0	0	0	0	0	0	0	0	0	0
Gekkonidae	<i>Hemidactylus mabouia</i>	0	0	0	0	0	0	0	0	0	0
Polychrotidae	<i>Anolis cristatellus</i>	4	6	148	150	126	3	2	89	101	7
Polychrotidae	<i>Anolis pulchellus</i>	0	0	4	3	26	0	0	9	0	0
Polychrotidae	<i>Anolis stratulus</i>	1	1	32	29	54	1	0	14	15	4
Sphaerodactylidae	<i>Sphaerodactylus gaigae</i>	0	0	37	53	0	0	1	56	45	0
Sphaerodactylidae	<i>Sphaerodactylus m. inigoï</i>	0	0	0	6	0	0	0	10	0	0
Dipsadidae	<i>Magliophis exiguus</i>	0	0	0	0	0	0	0	0	0	0
<b>Total individuals</b>		<b>8</b>	<b>9</b>	<b>299</b>	<b>314</b>	<b>231</b>	<b>7</b>	<b>6</b>	<b>267</b>	<b>217</b>	<b>11</b>
<b>Total species</b>		<b>3</b>	<b>3</b>	<b>7</b>	<b>8</b>	<b>6</b>	<b>4</b>	<b>4</b>	<b>8</b>	<b>5</b>	<b>2</b>

Table 3. Multi-Response Permutation Procedures (MRPP) analysis, pairwise comparison groups between the east and west areas and sampling periods (AM/PM) at the Vieques National Wildlife Refuge.

Pairwise comparison groups	T	A	P
East AM vs East PM	-7.56522368	0.05572458	0.00002789
East AM vs West AM	-6.75731409	0.04946586	0.00004406
East AM vs West PM	-13.19686065	0.11134303	0.00000028
East PM vs West AM	-22.36691570	0.16728953	0.00000000
East PM vs West PM	-18.31131353	0.13389258	0.00000000
West AM vs West PM	-16.18304321	0.12302278	0.00000000

A = 1 - (observed delta/expected delta)

A = 0 when heterogeneity within groups equals expectation by chance

A < 0 with more heterogeneity within groups than expected by chance

Table 4. Indicator Species Analysis (ISA), comparison East and West areas and sampling periods (AM/PM)

Species name	Pairwise groups	Importance Value (IV)	P *
<i>Anolis pulchellus</i>	East PM	69.3	0.0002
<i>Anolis stratulus</i>	West AM	50.7	0.0002
<i>Eleutherodactylus antillensis</i>	West PM	43.9	0.0002
<i>Eleutherodactylus cochranæ</i>	West PM	43.3	0.0002
<i>Osteopilus septentrionalis</i>	East PM	42.6	0.0002
<i>Anolis cristatellus</i>	East PM	31.6	0.0188
<i>Leptodactylus albilabris</i>	East PM	30.1	0.0016
<i>Sphaerodactylus gaigae</i>	West AM	29.9	0.0022
<i>Sphaerodactylus macrolepis inigoï</i>	East AM	15.5	0.1300
<i>Magliophis exiguus</i>	East AM	4.3	0.2278
<i>Hemidactylus mabouia</i>	East PM	3.4	1.0000

$p = (1 + \text{number of runs } \geq \text{observed}) / (1 + \text{number of randomized runs})$

Table 5. Species composition and distribution throughout 11 study sites of the Vieques National Wildlife Refuge, Vieques, Puerto Rico. Species were documented via visual encounter transect, drift fence arrays with pitfall and double ended funnel traps.

Species name	West area					East area						
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
<i>Eleutherodactylus antillensis</i>	X	X	X	X	X	X	X	X	X	X	X	X
<i>Eleutherodactylus cochranae</i>	X	--	X	X	X	--	--	--	--	X	X	X
<i>Leptodactylus albilabris</i>	--	X	X	X	X	X	--	X	X	X	X	X
<i>Osteopilus septentrionalis</i>	--	--	--	--	--	--	--	--	X	X	X	X
<i>Hemidactylus mabouia</i>	--	--	--	--	--	--	--	--	--	X	--	--
<i>Anolis cristatellus</i>	X	X	X	X	X	X	--	X	X	X	X	X
<i>Anolis pulchellus</i>	--	--	X	X	X	--	--	--	X	X	X	X
<i>Anolis stratulus</i>	X	X	X	X	X	--	--	--	X	X	X	X
<i>Sphaerodactylus gaigae</i>	--	X	X	X	--	--	--	--	--	X	X	X
<i>Sphaerodactylus m. inigo</i>	--	--	X	X	--	--	X	--	X	X	X	X
<i>Magliophis exiguus</i>	--	--	--	-	--	--	--	--	X	--	--	--

X = species documented; ( -- ) = species absence