Species Status Assessment Report
for the
Puerto Rican Harlequin Butterfly
(*Atlantea tulita)*

Version 1.5

Photo by Carlos Pacheco, U.S. Fish and Wildlife Service

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VERSION UPDATES

Version 1.4 is the final draft of the Puerto Rican Harlequin Butterfly Species Status Assessment developed by the U.S. Fish and Wildlife Service's Caribbean Ecological Services Field Office prior to formal peer review. Peer review comments were considered and addressed in this version, Version 1.5.

EXECUTIVE SUMMARY

We, the U.S. Fish and Wildlife Service (Service), developed a species status assessment (SSA) for the Puerto Rican harlequin butterfly (*Atlantea tulita*), a species endemic to the island of Puerto Rico. The Puerto Rican harlequin butterfly (PRHB) was added to the list of candidate species on May 31, 2011, when the Service published a 12-month finding indicating that listing was warranted but precluded by higher priority actions (76 FR 31282, May 31, 2011). This SSA will inform a new 12-month finding to determine whether the candidate species warrants listing.

The SSA process is intended to assess the viability of the species using the conservation biology principles 'the 3Rs' – resiliency, representation, and redundancy. In this SSA report we provide a summary of the species' biology at the individual, population, and species level; describe the factors that have led to its current status and those that are likely to influence its status into the future; assess the current and future health of individual populations given these influences; and describe the implications of predicted health and distribution on the 3Rs.

We identified five extant PRHB populations, each generally small in size, with less than 100 total individuals observed in any given year. Relative to historical conditions, the PRHB distribution is now fragmented among discrete remnants of native forest located in four ecological life zones in Puerto Rico. Land use in the species' range consists of urban developments, agriculture, and patches of native forest. The species can be positively or negatively influenced at local, landscape, and regional scales by factors like urban development (i.e., habitat modification, fragmentation), agricultural practices (i.e., grazing, haying), anthropogenic fires, pesticides, and climate change. An essential habitat feature for the PRHB is prickly bush (*Oplonia spinosa*), because it is used almost exclusively for egg laying and as a food source for the larval (caterpillar) life stage.

In this SSA, we consider the current condition of the PRHB based on its distribution, abundance, and those factors currently influencing the viability of the species. We evaluate the needs of the species in terms of the 3Rs and examine existing factors that are negatively and positively influencing the species (i.e., threats and existing voluntary or regulatory conservation efforts). Presently, we classified two (2) PRHB populations as having moderately high resiliency and three (3) as having moderately low resiliency. In the absence of highly certain population size or trend estimates, our classifications of resiliency rely heavily on habitat characteristics. The populations classified as having moderately high resiliency (Rio Abajo Commonwealth Forest and Rio Encantado Area) occur in habitats managed for conservation that are surrounded by forest and have a low probability of being affected by human activities. The three populations classified as moderately low (at Isabela, Quebradillas, and Camuy (IQC); Maricao Commonwealth Forest; and Susúa Commonwealth Forest) occur in areas where human activities

may negatively affect the species. Currently, we consider resiliency at the species level (rangewide) to be moderate.

To evaluate the future condition of the PRHB, we placed the broad spectrum of factors that influence species' viability into two main categories: habitat modifications and climate change. Next, we developed three future risk scenarios: (1) conditions staying the same as currently, with slight, insignificant changes in habitat modification, climate, and population sizes (Best Case Scenario); (2) conditions whereby impacts from development and climate change continue increasing at a moderate rate, with some decrease in population sizes (Most Likely Scenario); and (3) conditions whereby impacts from development and climate change continue increasing at a high rate and population sizes decreased substantially (Worst Case Scenario). Climate change was an important factor in our analysis of PRHB future condition, so we named the three scenarios to match the terminology used for the most recent climate change model for Puerto Rico. We chose 25 years as the time frame for the PRHB future conditions analysis because this time frame includes at least 25 generations, thus allowing adequate time to detect trends in populations and habitat conditions. Our predictions associated with this time frame are supported by existing predictive models regarding regional climate change. In particular, potential impacts associated with changing climatic conditions (e.g., estimates for precipitation and drought levels) are based on published climate model projections downscaled for Puerto Rico and the Virgin Islands.

Unless the Best Case Scenario transpires, we predict reductions in the 3R's, particularly redundancy and representation, over the next 25 years. Development for residential, commercial, and tourism uses, both within and adjacent to areas currently occupied by PRHB, will most likely increase over this time period, with attendant loss and degradation of suitable habitat, increased use of herbicides and pesticides, and greater risks of fires. These effects, both individually and collectively, have the potential to cause losses of not only annual reproductive cohorts, but also individual or multiple populations, thereby further reducing species viability. Although the adverse effects of development could be managed, the risk to PRHB viability imposed by forecast changes to climate will be more challenging to address. While the full ecological effects of these changes on the PRHB are unclear, it is likely that substantial changes in overall habitat and microhabitat (e.g., temperature, humidity) for a species whose ecology appears closely linked to specific current conditions (e.g., healthy *Oplonia spinosa* populations) will have negative effects on the PRHB.

At the end of our predictive time horizon (year 2045) at least three (3) of the current five (5) PRHB populations will most likely have been extirpated, with those remaining (i.e., IQC and Maricao) incurring reductions in resiliency. Those predicted to be lost are the populations at Río Abajo Commonwealth Forest, Rio Encantado area, and Susúa Commonwealth Forest, which represents approximately 25 percent of the currently known total population size. Because of concomitant population reductions in the remaining populations, the overall losses to the total PRHB population will be substantially greater than 25 percent, although impossible to accurately quantify at the current time.

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CHAPTER 1 – INTRODUCTION AND ANALYTICAL FRAMEWORK

The Puerto Rican harlequin butterfly (*Atlantea tulita*) is endemic to Puerto Rico. We, the U.S. Fish and Wildlife Service (Service), were petitioned to list the Puerto Rican harlequin butterfly (PRHB) in accordance with the Endangered Species Act of 1973 (Act) in February 2009, by the private citizen, Javier Biaggi-Caballero. On April 26, 2010, the Service published a 90-day finding that the petition presented substantial scientific or commercial information indicating that listing may be warranted for the PRHB (75 FR 21568, April 26, 2010). On May 31, 2011, the Service published a 12-month finding indicating that listing this species was warranted but precluded by higher priority actions (76 FR 31282, May 31, 2011). Upon publication of that 12-month finding, the PRHB was added to the candidate species list. A review of the status of this candidate species has been initiated to determine if listing is still warranted. Thus, we conducted a Species Status Assessment (SSA) to compile the best available data regarding the species' biology and factors that influence the species' viability. The PRHB SSA Report is a summary of the information assembled and reviewed by the Service, and incorporates the best scientific and commercial data available. This SSA Report documents the results of the comprehensive status review for the species and serves as the underpinning of the Service's forthcoming decision (12-month finding) on whether the species warrants protection under the Act.

The SSA framework (U.S. Fish and Wildlife Service (USFWS) 2016, entire) is intended to be an in-depth review of the species' biology and threats, an evaluation of its biological status, and an assessment of the resources and conditions needed to maintain long-term viability. The intent is for the SSA report to be easily updated as new information becomes available and to support all functions of the Ecological Services Program of the Service, including Candidate Assessment, Listing, Consultations, and Recovery. As such, the SSA report will be a living document that may be used to inform Endangered Species Act decisions, such as listing, recovery, Section 7, Section 10, and reclassification decisions (the latter four decision types are only relevant should the species warrant listing under the Act). Therefore, we developed this SSA report to summarize the most relevant information regarding life history, biology, and considerations of current and future risk factors facing the PRHB. In addition, we forecast the possible response of the species to various future risk factors and environmental conditions to formulate a complete risk profile for the species.

The objective of this SSA is to describe the viability of the PRHB based on the best scientific and commercial information available. Based on this information, we assess what the species needs to support viable populations and its current condition in terms of those needs, and we forecast the species' conditions under plausible future scenarios. This SSA report considers past, ongoing, and plausible future changes in the environment to help us understand what factors drive the viability of the species.

For the purpose of this assessment, we define **viability** as the ability of a species to sustain populations in the wild. Viability is not a specific state, but rather a continuous measure of the likelihood that the species will sustain populations over time (USFWS 2016, p. 9). Using the SSA framework (Figure 1-1), we consider what the species needs to maintain viability by characterizing the status of the species in terms of its **resiliency**, **representation**, and **redundancy** (USFWS 2016, entire).

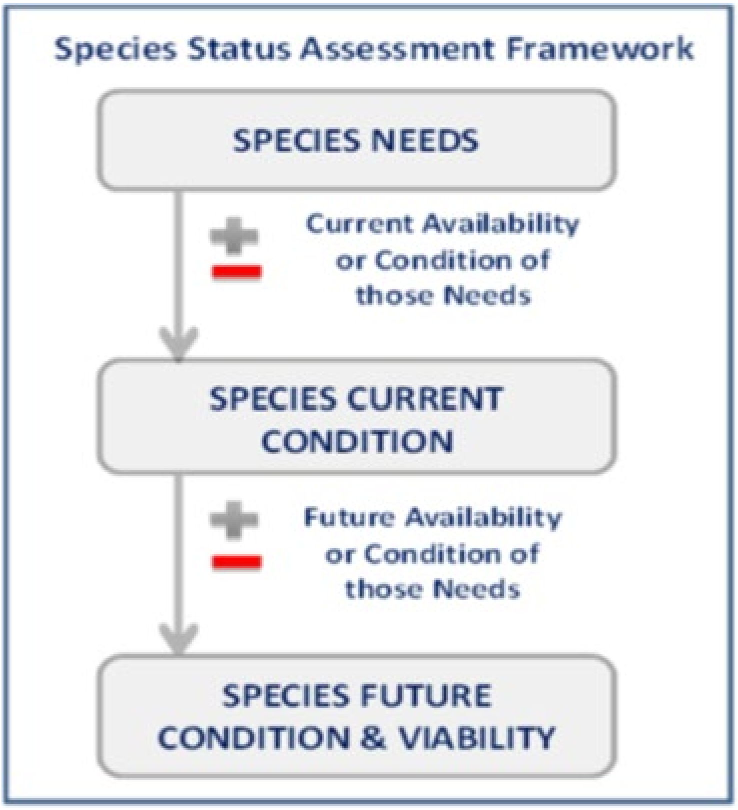


Figure 1-1. Species Status Assessment Framework

Resiliency describes the ability of a population to withstand stochastic disturbance. Stochastic events are those arising from random factors such as weather, flooding, or fire. Resiliency is positively related to population size and growth rate and may be influenced by connectivity among populations. Generally speaking, populations need enough individuals, within habitat of adequate area and quality, to maintain survival and reproduction in spite of disturbance. Resiliency is measured using metrics that describe population condition and habitat quality.

Representation describes the ability of the species to adapt to changing environmental conditions over time. Representation can be measured through the genetic diversity within and among populations and the ecological diversity (also called environmental variation or diversity) of populations across the species' range. Theoretically, the more representation the species has, the higher its potential of adapting to changes (natural or human caused) in its environment. In the absence of genetic data, we used the number of life zones harboring resilient populations of the PRHB to assess representation.

Redundancy describes the ability of a species to withstand catastrophic events. A catastrophic event is defined here as a rare, destructive event or episode involving multiple populations and occurring suddenly. Redundancy is about spreading risk among populations, and thus, is assessed by characterizing the number of resilient populations across a species' range. The more resilient populations the species has distributed over a larger area, the better the chance is that the species can withstand catastrophic events. For the PRHB, we used the number of known populations to measure redundancy.

To evaluate the biological status of the PRHB both currently and into the future, we assessed a range of conditions to allow us to consider the species' resiliency, redundancy, and representation (together, the 3Rs). This SSA report provides a thorough assessment of the species' biology and natural history and assesses demography, stressors, and limiting factors in the context of determining the viability and risk of extinction for the species.

Importantly, this SSA report does not result in, nor predetermine, any decisions by the Service under the Act. In the case of the PRHB , the SSA report does not determine whether this species warrants protections of the Act, or whether it should be proposed for listing as a threatened or endangered species under the Act. That decision will be made by the Service after reviewing this document, along with the supporting analysis, any other relevant scientific information, and all applicable laws, regulations, and policies. The results of the decision will be announced in the *Federal Register*. The contents of this SSA Report provide an objective, scientific review of the available information related to the biological status of the PRHB.

CHAPTER 2 –NEEDS OF INDIVIDUALS: LIFE HISTORY AND BIOLOGY

This chapter provides a summary of basic ecological and biological information about the PRHB, including its taxonomy, physical description, life history, and habitat. We then outline the resource needs of individual. We evaluate the life history characteristics to determine the specific biological or environmental resources that are relevant for the species to complete its life cycle and ensure its survival into the future. This information is important for a thorough understanding of the resources the species needs to (1) carry out its life history; (2) have the population persist over time such that it can withstand stochastics events; and (3) have sufficient healthy population distributed such that catastrophic event will not cause the species to go extinct and will also allow it to adapt to changing environmental conditions. These biological and environmental resources needs are later used to compare against relevant influences (see Chapter 4), which helps provide a risk profile for the PRHB.

2.1 Taxonomy

Atlantea tulita is a valid species belonging to the family Nymphalidae. The currently accepted taxonomy ranking for this butterfly is as follows:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Lepidoptera (Linneaus 1758)
Group: Rhopalocera (Boisduval 1840)
Super-Family: Papilionoidea (Dyar 1902)
Family: Nymphalidae (Swainson 1827)
Sub-Family: Nymphalinae (Doubleday 1845)
Tribe Melitaeine (Newman 1870)
Genus: *Atlantea* (Higgins 1958)
Species: *Atlantea tulita* (Dewitz 1877)

Original: *Synchloe tulita* (Dewitz 1877, p. 238); Synonimia: *Coatlantona tulita* (Moschler 1891, p. 96); *Chlosyne perezi tulita* (Forbes 1928, p. 98; Comstock 1930, p. 449).

Currently, the genus *Atlantea* (Higgins 1958), is represented by a single species on each of the Greater Antilles (Figure 2-1; Higgins 1981, p. 174). That is, *Atlantea perezi* (Herrich-Schaffer 1862) in Cuba, *Atlantea pantoni* (Kaye, 1906) in Jamaica, *Atlantea cryptadia* (Sommer & Schwartz, 1980) in Hispaniola, and *Atlantea tulita* (Dewitz 1877) in Puerto Rico (Carrión-Cabrera 2003, p. 1).

The butterfly, *Atlantea tulita,* has been referred to by different common names in the literature. For example, the species has been named as the Puerto Rican harlequin butterfly or the Puerto Rican checker-spot butterfly, but is also known as "La Quebradillana" because the species was first discovered in the municipality of Quebradillas. For the purpose of this SSA, we refer to the common name as the Puerto Rican harlequin butterfly (hereafter, PRHB).

Figure 2-1. Map showing the distribution of the genus *Atlantea* through the Caribbean Region.

2.2 Species Description

The PRHB is a medium size butterfly. The species has a wingspan of about 5.1 to 6 centimeters (cm) (2 to 2.5 inches (in)) wide and is characterized by its orange, brownish-black and beige coloration patterns (Figures 2-2 and 2-3). The butterfly is brownish-black at the thorax area with deep orange markings. The male's abdomen is brownish-black on the dorsal side and has orange and brown bands on the ventral side. The female's abdomen is brownish-black with white bands. Wings are largely brownish-black with sub-marginal rows of deep orange spots and beige cells. The dorsal view of the forewings and the hind-wings, the outer margins are brownish-black. The coastal margin is deep orange with brownish-black markings. The inner margin is brownish-black with some deep orange markings at the half basal wing. The hind wing has a wide black border enclosing a set of reddish-bronze sub-marginal points. As a member of the checker-spot butterfly group, rows of deep orange dots (or cells) is a typical pattern on the species' brownish-black wings. The ventral sides of the forewings are similar to the dorsal sides of the forewings, and ventrally the hindwings are brownish-black with orange basal spots, a complete postdiscal beige band with a band of reddish spots distally, and sub-marginal white half-moons.

The chrysalis (pupa from which the butterfly (adult, or imago) emerges) of the PRHB is black, with orange and white dashes, and yellow pimples (Biaggi-Caballero 2009, p. 4) (Figure 2-4). Chrysalis size is around 3 cm (1.2 in).

Figure 2-2. Photos showing the dorsal (top) and ventral (bottom) coloration patterns observed in *Atlantea tulita* (Dewitz 1877). Male (left) and female (right). Photo downloaded from https://www.butterfliesofamerica.com.

Figure 2-3. Male (left) and female (right) *A. tulita*. The abdomen of the male is a deep orange color with bands, and the abdomen of the female is white with black bands. Source: Carlos Pacheco, Service.

The PRHB caterpillar (larva) is dark orange with a brownish-black to black, thin sub-lateral line, over a thin line of white intermittent dots crossing the body from the head to anal plate (Figure 2-5). The larva is less than 4.76 millimeter (mm)(0.19 in) in first instar (growth stage between molts) and about 55.8 mm (2 in) in the fifth instar (C. Pacheco, Service, 2018, personal observation). The body of the larva has spines with hairs in each body segment (Figure 2-5).

Figure 2-4. Chrysalis of *Atlantea tulita*. Photos by C. Pacheco, Service.

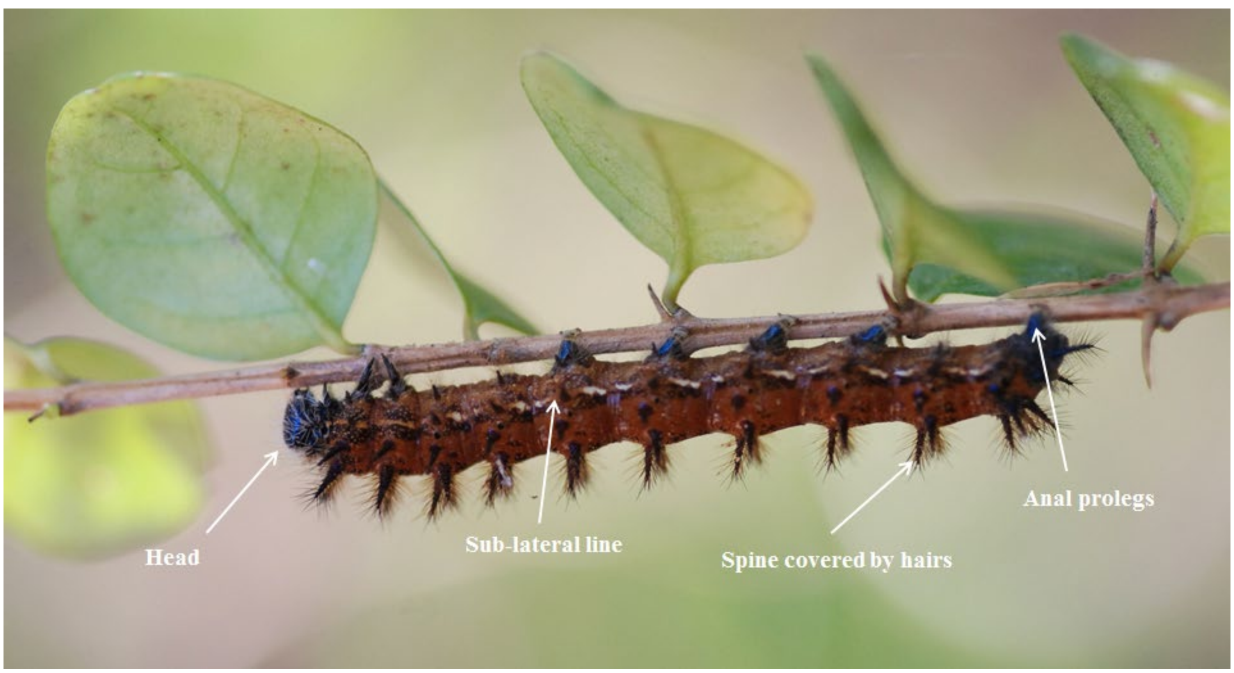


Figure 2-5. *Atlantea tulita* caterpillar. Photo by C. Pacheco, Service.

The eggs of the PRHB are greenish oily spheres, with a yellowish incipient crown (Figure 2-6).

Figure 2-6. Photo (left) showing the yellowish crown on the eggs laid by *Atlantea tulita* on *Oplonia spinosa*. Photo (right) showing the first instars of the *Atlantea tulita*. Photo by C. Pacheco, Service, 2011.

2.3. Life History

Most of what is known about PRHB life history, demography and behavior comes from field observations, information gathered from other species from the same family, and expert opinions.

2.3.1 Life Cycle

The life cycle of the PRHB includes four distinct anatomical stages: egg, larva (caterpillar, with several size phases called instars), chrysalis, and imago (adult). It is a general consensus among the species' experts (A. Morales and E. Estremera, Liga Ecologica Quebradillana; H. Torres, former Assistant Professor from the University of Puerto Rico, Mayagüez Campus; and C. Asencio, former professor Universidad Católica de Ponce) that the life cycle of the PRHB (Figure 2-7) from egg to imago in the wild may take around 125 days (Second Technical Meeting Puerto Rican Harlequin Butterfly Working Group, November 3, 2018). These experts also agree that the length of the life cycle can be affected by factors such as temperature and humidity, particularly at the caterpillar stage.

2.3.2 Dispersal, Mating, and Food Sources

PRHB dispersal and mating behavior has not been thoroughly studied. The butterfly flies slowly and is weak and fragile; thus, the species is considered a poor disperser (Carrión-Cabrera 2003, p. 51). However, Monzón (2007, p. 42) found that the butterfly can disperse up to 1,026 meters (m) (3366.1 feet (ft)), approximately 1 kilometer (km) (0.6 mile (mi)) from one breeding site to another. Additionally, the species has specific ecological requirements for reproduction and its dispersion is apparently limited by the monophagus habit of the first instar of the larvae, which feeds only on prickly bush (*Oplonia spinosa*) (Carrión-Cabrera 2003, p. 40; Biaggi-Caballero 2009, p. 4). Mating behavior has been rarely documented. For other species in the family Nymphalidae, the male grasps the female in flight and brings her to a surface, such as a leaf (Figure 2-8) or the ground, where mating occurs. Carrion-Cabrera (2003, p. 60) estimated the sex ratio of the PRHB as 2.67 males per female. It is not well known if the PRHB mates during a particular month of the year or year-round. However, all life stages of the PRHB are observed year-round, suggesting that mating and oviposition may occur at any time during the year (Figure2-9).

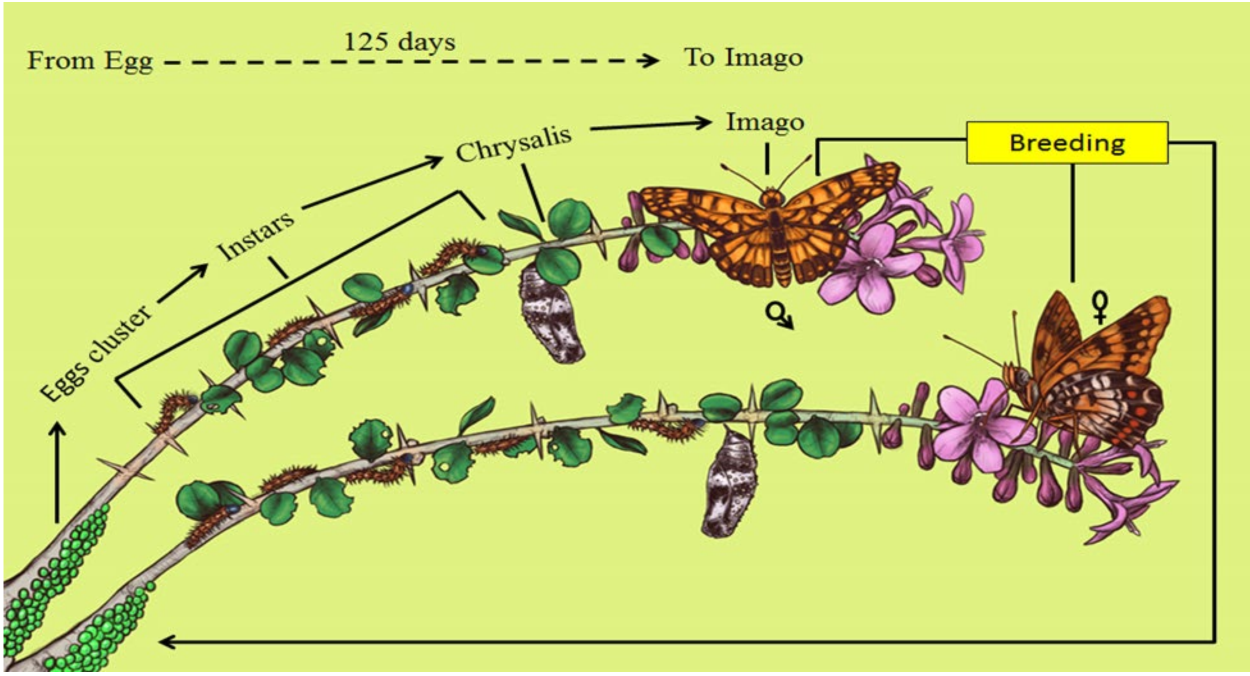


Figure 2-7. Conceptual diagram of the Puerto Rican harlequin life cycle.

Figure 2-8. Photo of Puerto Rican Harlequin butterfly mating. Photo by José Chabert (President of "Fundanción EL Pastillo") at El Pastillo in the municipality Isabela.

Females are multivoltine ovipositors (they produce several broods in a single season) (Biaggi-Caballero 2009, p. 2; 76 FR 31282, May 31, 2011, p. 31283). Eggs and larvae have been found almost exclusively on prickly bush (*Oplonia spinosa*) (Figures 2-10 and 2-11). The female lays the eggs in rows singly or in pairs, on the underside of tender twigs of the host plant. The species uses the tender vegetative branches of new growth of the host plant for bearing its eggs and feeding during the larval stages (Carrion-Cabrera 2003, p. 40; Biaggi-Caballero and Lopez 2010, p. 2). New growth of *O. spinosa* is observed a few days after rain events, being more abundant during the wet season (from April to November).The female of the PRHB can lay between 50 to 140 eggs in about 45 minutes (Carrion-Cabrera 2003, p.38; Biaggi-Caballero 2009, p. 4). During this process the female appears to be undisturbed by the presence of humans or any other threats (Barber 2018, p. 2).

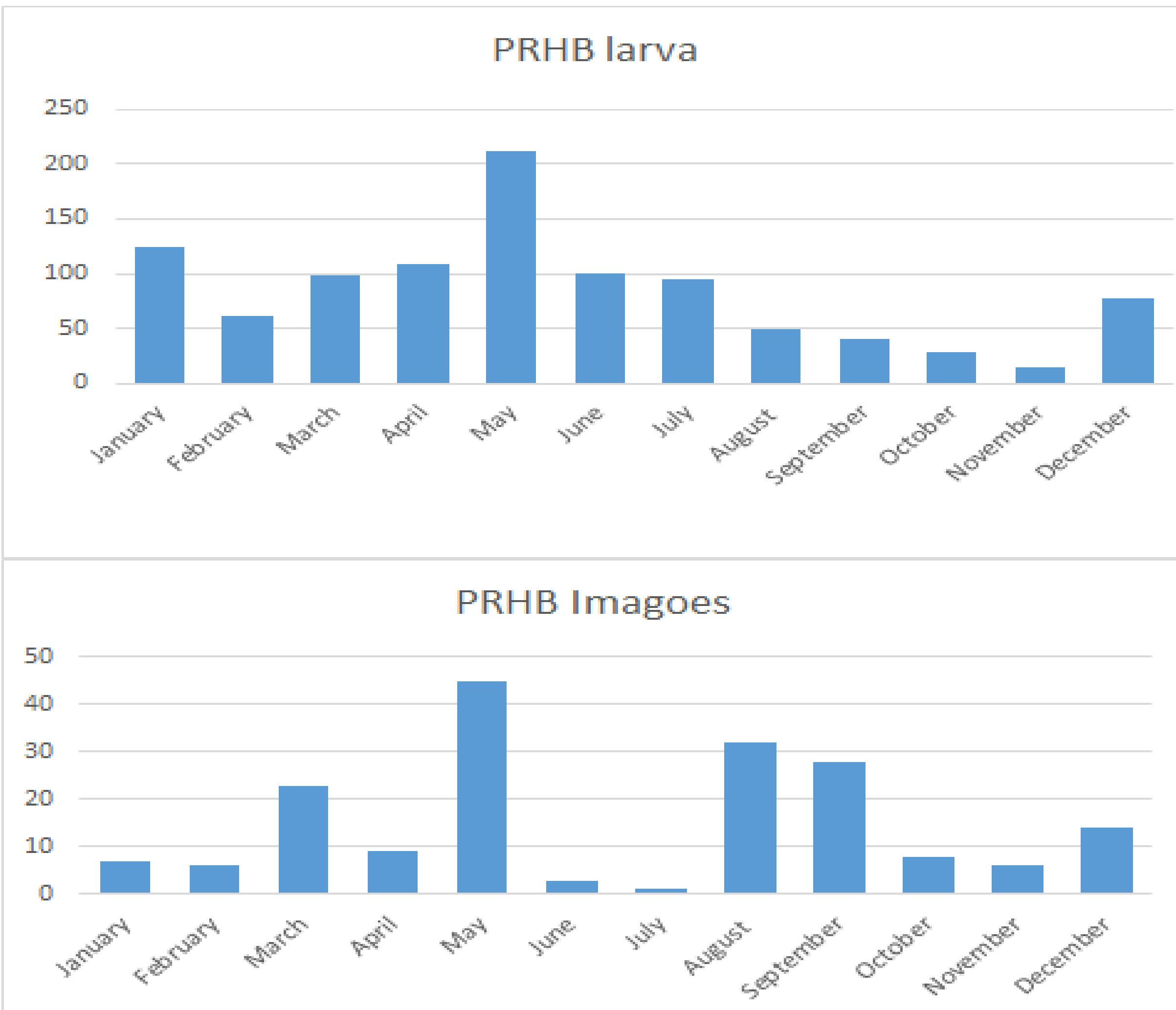


Figure 2-9. Number of observed PRHB larvae (top) and imagoes (bottom) per month throughout the year. Based on information provided by Carrion-Cabrear 2003, Monzon 2007, Biaggi-Caballero 2010, and Barber 2018.

Broods of the PRHB generally contain 50 to 150 eggs, with an average of 102 eggs per brood (Carrion-Cabrera 2003, p.38). The time to egg eclosion and viability (hatching success) rate have not been determined. After egg eclosion, the first instars devour the egg shells and then begin feeding from the most tender parts of the host plant (Biaggi-Caballero and López 2010, p.2). As the first instar matures, PRHB larvae crawl to the older and woody part of the host plant eating any new growth, including leaves and stems.

Figure 2-10. Left: female *Atlantea tulita* laying eggs on the host plant, prickly bush (*Oplonia spinosa)*. Right: eggs of *Atlantea tulita* laid on the new growth (tender part) of *O. spinosa* branches. Photo by José Vargas, 2018.

Figure 2-11. Prickly bush (*Oplonia spinosa*) (left), Family Acanthaceae; endemic to several Caribbean islands and widely distributed in Puerto Rico. Eggs (right) found on prickly bush Source: Willie Hernandez, Liga Ecológica Quebradillana, 2009.

Although the PRHB is believed to be a specialist because of its monophagous habit of feeding only on *O. spinosa*, recently Barber (2016, p. 9) documented a PRHB larva feeding on *Odontonema cuspidatum* (commonly known in Puerto Rico as "coral de jardín") in Quebradillas (Figure 2-12). Like *O. spinosa*, *O. cuspidatum* is in the family Acanthaceae, but it is native to Mexico and has been introduced to the West Indies as an ornamental shrub (Axelrod 2011, p. 50). In addition, Morales and Estremera (2018, unpublished data) found that the PRHB caterpillar also feeds on *Justicia mirabiloides* (commonly known as West Indian water-willow; or in Spanish as papayo montuno). *Justicia mirabiloides*, which is also in the family Acanthaceae, is a perennial herb native to Puerto Rico and the Virgin Islands. These rare observations of the PRHB caterpillar feeding on plants other than *O. spinosa* were of later instars (possibly 4th or 5th instar). Therefore, it is not known whether the first instar can use other plant species as a food source. Regardless, given the paucity of observations of feeding on other plant species, *O. spinosa* is an essential PRHB food source.

When the caterpillar is fully grown, it makes a button of silk which it uses to fasten its body to a leaf or a twig. Then, the caterpillar's skin comes off the final time, revealing the chrysalis. In the wild, the chrysalis is more often found attached to branches of plants located close to the host plant, but it has been observed attached to dried twigs of the host plant (Biaggi-Caballero 2009, p. 3).

Adult PRHBs have been observed feeding on flowers of several native trees: *Bidens pilosa*, *Bourreria suculenta*, *Bourreria virgata*, *Bursera simaruba*, *Citharexylum fruticosum*, *Coccoloba uvifera*, *Coccoloba diversifolia*, *Coccoloba swartzii*, *Coccoloba costata*, *Coccoloba pubescens*, *Croton rigidus*, *Erithalis fruticosa*, *Guettarda ovalifolia*, *Justicia mirabiloides*, *Lantana camara*, *Lantana involucrata*, *Leucaena leucocephala*, *Oplonia spinosa*, *Paulinia pinnata*, *Pisonia horneae*, *Pisonia subcordata*, *Stigmaphyllon emarginatum*, *Tabebuia heterophylla*, and *Vernonia albicaulis* (76 FR 31282, May 31, 2011, p. 31283; Chabert 2015, p. 2; Barber 2018, p. 3; Vargas 2019, p. 14).

Figure 2-12. Larva of *Atlantea tulita* feeding on *Oplonia spinosa* (prickly bush; left and center) and on *Odontonema cuspidatum* ("coral de jardín" right).

2.4. Habitat

The PRHB host plant for egg laying and larval feeding, *O. spinosa,* is a common tropical shrub that is widely distributed in Puerto Rico. According to Lioger (1997, p. 42), *O. spinosa* is a shrub of variable habits that occurs on hillsides and in woods and thickets, at lower and middle elevations in Puerto Rico, Culebra, Vieques, Bahamas and West Indies. Water and nectar sources for adult PRHBs may vary according to the life zone and habitat type. All the sites where the PRHB occurs have a close (within a 1 km radius) water source (e.g., creek, river, pond, among others).

In addition to *O. spinosa*, the a list of the woody plant species known to occur in areas inhabited by the PRHB is provided in appendix I. In some areas were the PRHB occurs, federally listed species such as *Daphnosis helleriana*, *Schoepffia arenaria* and *Ottoschulzia rhodoxylum* are present (Morales and Estremera 2018, p. 1; Vargas 2019, p. 3). Other rare species that co-occur with the PRHB in some areas include *Minikara pleeana*, *Pisonia woodburyana*, *Drypetes ilicifolia* and *Tabebuia karsoana* (Morales and Estremera 2018, p. 1). The presence of these plant taxa suggests that the areas where the PRHB occurs are relicts of mature forest that might have survived the massive deforestation of 19^{th} and early 20th centuries (Morales and Estremera 2018, p. 1).

2.5 Summary of Individual Needs

As discussed above, individuals of the PRHB have a variety of resource needs depending on life stage. These needs are summarized in Table 2-1.

Table 2-1. Resources needed by the Puerto Rican harlequin butterfly to complete its life cycle.

Resources needed by each PRHB life stage		Resource Function	Information Sources
Eggs			
	Oplonia spinosa (host plant) with tender vegetative new growth, broadleaf plants, dry-mesic habitat	Breeding	Carrion-Cabrera 2003; Monzón 2007; Biaggi-Caballero 2010;
Caterpillar and chrysalides			
	Food source: *Oplonia spinosa* (host plant), *Odontonema cuspidatum*, *Justicia mirabiloides*. Temperature and relative humidity may be important for larval survival.	Feeding	Carrion-Cabrera 2003; Monzón 2007; Biaggi-Caballero 2010; Barber 2016; Morales and Estremera 2018
	Forested habitat: mosaic of forested habitat with canopy cover between 50 to 85 percent, average canopy height of 20 feet, and plant host cover of more than 30 percent.	Foraging, Sheltering, Migration, Dispersal	Morales and Estremera 2018; Vargas 2019
Adult (imago)			
	Food source: *Bidens pilosa, Bidens urbanii, Bourreria suculenta, Bourreria virgata, Bursera simaruba, Chromolaena sinuate, Coccoloba uvifera, Coccoloba diversifolia, Coccoloba swartzii, Coccoloba costata, Coccoloba pubescens, Croton rigidus, Erithalis fruticosa, Guettarda ovalifolia, Lantana camara, Lantana involucrata, Leucaena leucocephala, Oplonia spinosa, Paulinia pinnata, Pisonia horneae, Pisonia subcordata, Randia aculeata, Stachytarpheta jamaicensis, Vernonia albicaulis*.	Feeding, Sheltering, Migration, Dispersal	Carrion-Cabrera 2003; Monzón 2007; Biaggi-Caballero 2010; Barber 2016; Morales and Estremera 2018; Vargas 2019
	Forested habitat: mosaic of forested habitat with canopy cover between 50 to 85 percent, average canopy height of 20 feet, forested corridor between suitable breeding sites (with plant host covering more than 30 percent). Water source.	Foraging, Sheltering, Migration, Dispersal	Morales and Estremera 2018; Vargas 2019

CHAPTER 3. DISTRIBUTION, AND POPULATION AND SPECIES NEEDS.

In this chapter, we summarize the available information on the distribution and populations of the PRHB, the habitat where the species occurs, and those needs that may influence the viability of the species. We first identify the species' historical and current distribution throughout its entire range. Next, we define what we consider as a population and its structure, and we estimate population sizes. Finally, we characterize the needs of the species in terms of population resiliency and species' representation and redundancy (the 3Rs).

3.1. Historical Range

The PRHB was first collected and described from the karst hills in the municipality of Quebradillas in northern Puerto Rico (Dewitz 1877, p. 241). Later, the species was reported by William P. Comstock (1930, p. 449) in the municipality of Arecibo (northern Puerto Rico) and also in Quebradillas, and in Tallaboa, a location between the municipalities of Guayanilla and Peñuelas in the southern karst of the Island (Figure 3-1). The northern and southern karst regions are separated from each other by the central mountain range (Cordillera Central) that extends across the interior of Puerto Rico from east to west. Early observations indicated the PRHB occurred at low elevations in coastal areas (Gundlach 1891, p. 125). Much later, in 2003, the species was found at higher elevations in the municipalities of Maricao and Sabana Grande (Figure 3-1), both located within the west-central volcanic region (Carrion 2003, p. 32, Biaggi 2009, p. 3). These reports expanded the known range of the species from the coastal and karst area to the volcanic region, and from low lying coastal areas to elevations around 867 m (2,845 ft) above sea level.

Figure 3-1. Map showing the historical distribution of the Puerto Rican harlequin butterfly.

Many records of PRHB historical locations are from anecdotal reports. Therefore, precise information about locations where the species has been collected or sighted is limited and some records may not be accurate. By 2011, the PRHB was considered endemic to the northern karst region and the west-central section of Puerto Rico (Figure 3-1), since it has not been found in other areas of the Island or in other islands in the Caribbean (76 FR 31282, May 31, 2011).

3.2 Current Range

Since the PRHB was added to the candidate species list (76 FR 31282, May 31, 2011), search efforts for the species have been concentrated in its historical collection sites and other areas that harbor suitable habitat in Puerto Rico. Thus, for the purpose of this SSA we consider the species' occurrence as the area where the species has been found in all of its life stages (i.e., imago, larva and egg). Presently, this butterfly is only known to occur in the northern karst region and in the west-central volcanic-serpentine region (Perez-Asso et al. 2009, p. 94; Barber 2018, p. 2, Morales and Estremera 2018, unpublished data, p. 2) (Figure 3-2).

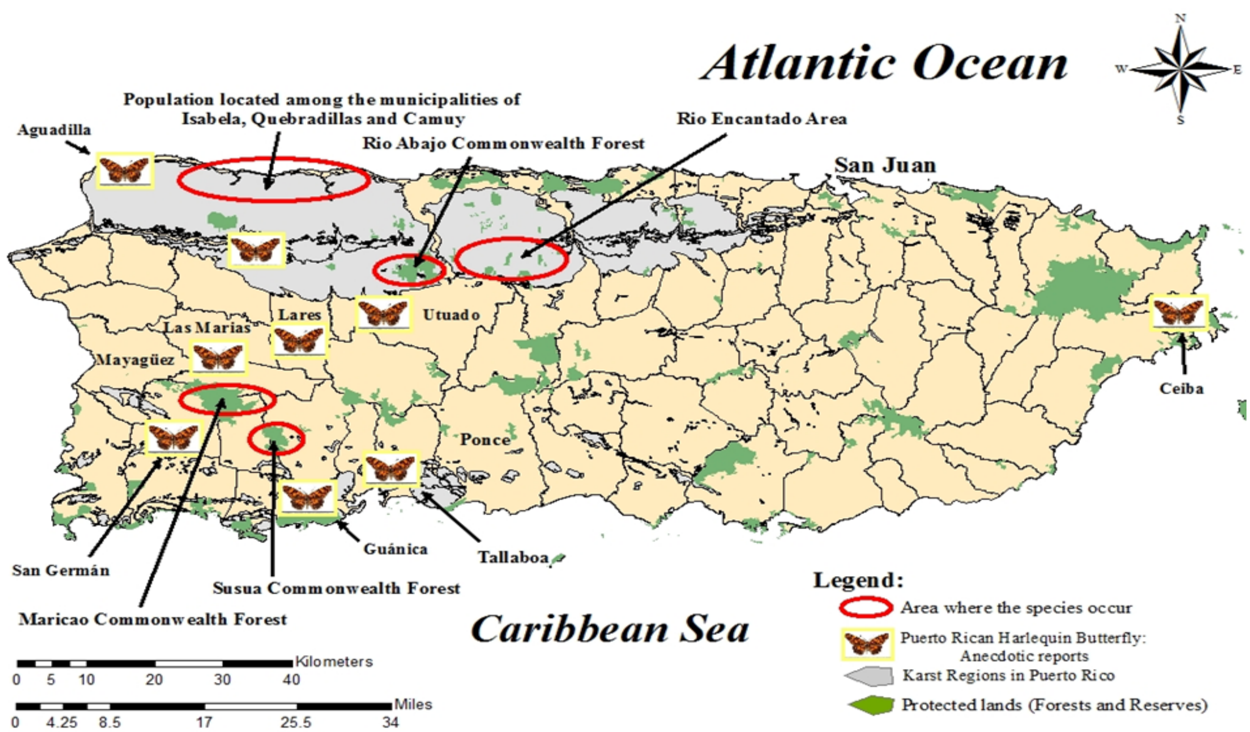


Figure 3-2. Map showing the areas where the Puerto Rican harlequin butterfly populations occur and anecdotal reports of the species in Puerto Rico.

Unfortunately, the fate of the PRHB in the southern karst region is unknown because the species has not been found since 1926 (Biaggi-Caballero and López 2010, p. 4). However, the PRHB has been anecdotally reported (adults, but not other life stages) in other regions, including the municipalities of Aguadilla, Barceloneta, Ciales, Florida, Luquillo, Ceiba, Guánica, San Germán, Las Marias, and Lares (Rivera Declet 2015, p. 20). Some of these anecdotal reports have been confirmed, while others need to be confirmed.

The PRHB occurs in four life zones: subtropical moist forest on limestone-derived soil, in the northern coastal cliff in Quebradillas (Helmer et al 2002, p. 169); subtropical moist forest on limestone-derived soil in the northern karst region; subtropical wet forest on serpentine-derived soil in the Maricao Commonwealth Forest; and subtropical dry/moist forest on serpentine-derived soil in the Susúa Commonwealth Forest (Ewel and Whitmore 1973, p. 25; Helmer et al 2002, p.169) (Figure 3-3). Although the species has not been detected for many years in the subtropical dry forest in southern karst, this region should be considered as potentially suitable habitat for the PRHB because it harbors *O. spinosa* and some of the plant species that the butterfly stage feeds upon (Carrion 2003, p. 31).

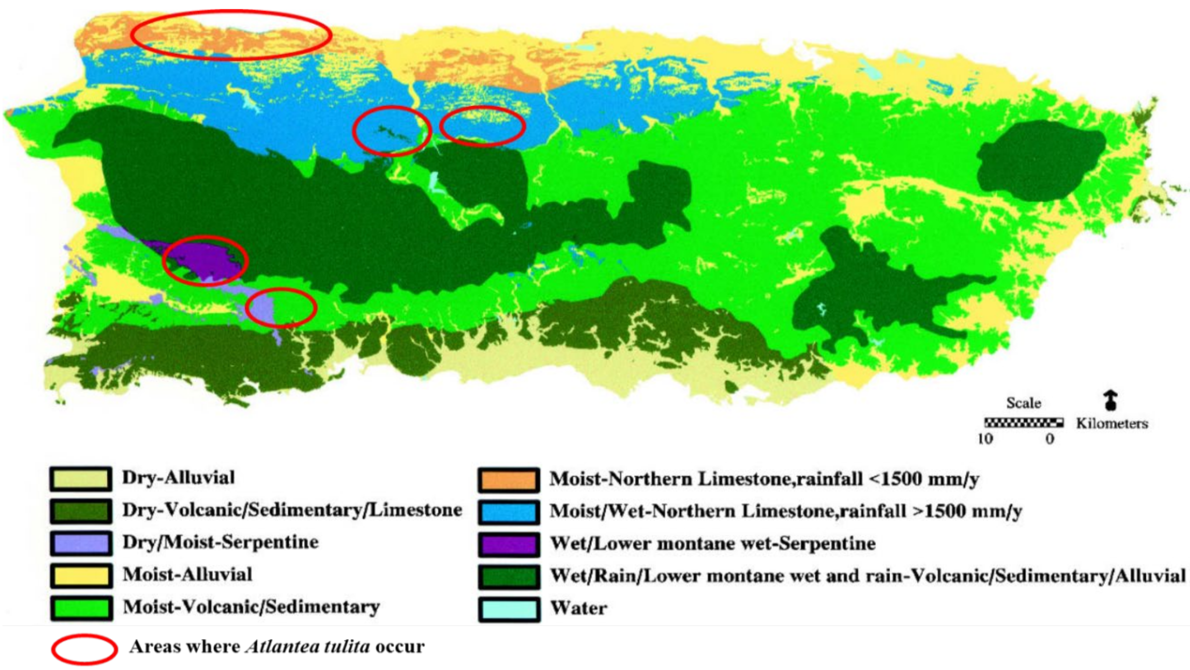


Figure 3-3. Map showing the locations where the Puerto Rican harlequin butterfly occurs in relation with the different forest type and life zones in Puerto Rico. (Helmer et al 2002, p. 169).

3.3 Current Distribution and Population Structure

In this SSA we identified five areas currently occupied by the PRHB that we refer to as a population, three in the northern karst region and two in the central-western volcanic-serpentine region (Figure 3-2). Conceptually, we treat each of the five populations as a metapopulation (Table 3-1), or a discrete population composed of local populations (subpopulations) with individuals that can move infrequently from one subpopulation to another (Hanski and Gilpin 1991, pp. 4 and 7). Genetic data to determine true population structure are lacking. However, the gaps in suitable habitat between the metapopulations, as we have defined them, coupled with the low dispersal capability (approximately 1 km (0.6 mi)) of the PRHB, suggests there is little to no interaction between the metapopulations.

Table 3-1. Puerto Rican harlequin butterfly regions of occurrence, metapopulation locations, and number of subpopulations per municipality.

Region of Puerto Rico	Metapopulation Location	Number of subpopulations	Number of subpopulations per Municipality (Municipality)	(Amount)
Northern Karst Region	Isabela, Quebradillas, and Camuy (IQC)	13	Isabela Quebradillas Camuy	6 6 1
	Río Encantado	3	Arecibo Florida Ciales	1 1 1
	Río Abajo Commonwealth Forest	1	Arecibo	1
West-central Volcanic-Serpentine Region	Maricao Commonwealth Forest	3	Maricao San Germán	1 2
	Susúa Commonwealth Forest	2	Sabana Grande Yauco	1 1
Southern Karst Region	Tallaboa	unknown	Guayanilla Peñuelas	unknown

3.3.1 Northern Karst Region Populations

In the northern karst region of Puerto Rico, the PRHB is known to occur in three areas (Figure 3-2): along a coastal cliff in the municipalities of Isabella, Quebradillas, and Camuy (Morales and Estremera 2018, unpublished data, p. 1, Barber 2019, p. 2); In the area of Río Encantado in the municipality of Florida, Ciales and Arecibo (Morales and Estremera 2018, unpublished data, p. 1); and at the Río Abajo Commonwealth Forest, between the municipalities of Arecibo and Utuado (Monzon 2007, p. 51; Morales and Estremera 2018, p. 1).

<u>3.3.1.1 Isabela, Quebradillas and Camuy Metapopulation</u>

In the area of Isabela, Quebradillas, and Camuy (IQC), the species' distribution has expanded since the PRHB was added to the candidate species list (76 FR 31282, May 31, 2011). At that time, the PRHB was known only to occur in four locations within the IQC area: (1) At "El Tunel de Guajataca" in Isabela, (2) "El Merendero" in Quebradillas, (3) "Puente Blanco" in Quebradillas, and (4) "Puerto Hermina" in Quebradillas. Currently, the species is known to occur in 13 areas within an approximately 400 hectare (ha) (988 acre (ac)) strip of forested habitat on a coastal cliff that extends along the municipalities of Isabela, Quebradillas, and Camuy (Morales and Estremera 2018, unpublished data, p. 1, C. Pacheco, Service, 2018, unpublished data). The species' range is delimited on the east by the community La Yeguada and Membrillo in Camuy, on the west by the community Villa Pesquera and Pueblo in Isabela, on the north by the Atlantic Ocean, and on the south by State road PR-2, the Royal Isabela Golf Course and some deforested areas utilized for agricultural practices such as cattle grazing (Figure 3-4). In those 13 areas, all life stages of the species (i.e., imago, egg, larva, chrysalis, and adults), and the species' host plant, have been found in 115 sites (Figure 3-4).

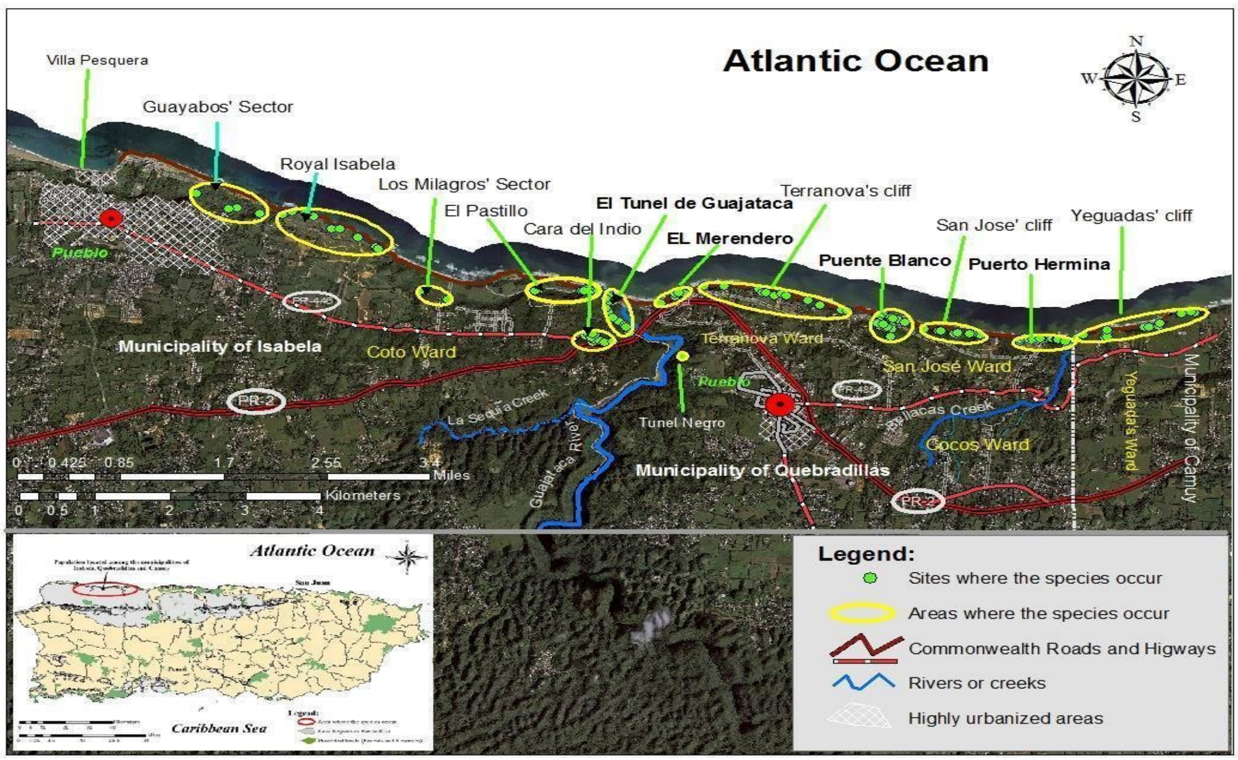


Figure 3-4. Map showing the distribution of the Puerto Rican harlequin butterfly along the municipalities of Isabela, Quebradillas and Camuy.

In the municipality of Quebradillas, the PRHB currently occurs in 5 areas scattered along the coastal forested cliff that extends from El Merendero in Terranova ward through Puente Blanco, to Puerto Hermina in San José Ward. Also, it is found in the forested areas in Puente Blanco and Puerto Hermina. The PRHB also currently occurs farther inland in Quebradillas at Tunel Negro (Figure 3-4). Within this range, all life stages of the species and its host plant have been observed in 55 sites (Morales and Estremera 2018, unpublished data, entire).

In the municipality of Isabela, the PRHB currently occurs in 6 areas scattered along the forested cliff that extends from El Tunel de Guajataca through El Pastillo beach and Royal Isabela Golf Course, to the Villa Pesquera at Coto Ward (J. Charbert, Fundación El Pastillo, 2018, personal communication; A. Morales, Liga Ecologica Quebradillana, 2018, personal communication; Figure 3-4). Within these areas, all stages of the butterfly and the host plant have been observed in 46 sites. Additionally, in the municipality of Camuy, the species currently occurs along the forested cliff from Puerto Hermina to the community La Yeguada. Within this area, all stages of the species and the host plant have been observed in 14 sites.

3.3.1.2 Río Encantado Metapopulation

The Río Encantado area is located to the east of the Arecibo River, within the municipalities of Arecibo, Manatí, Florida, and Ciales in the north-central section of the Island, approximately 50 km (31 miles (mi)) southeast from Quebradillas. This area comprises over 6,474.9 ha (16,000 ac) considered by Federal and Commonwealth conservation agencies as mature native secondary forest, holding the largest tract of continuous forest cover in all Puerto Rico (www.paralanaturaleza.org/en/rio-encantado-eng). Presently, the PRHB is known to occur in three (3) areas scattered through the Río Encantado (Figure 3-5; Morales and Estremera 2018, unpublished data, p. 1). Within these areas, all life stages of the species and the host plant have been observed in 8 locations. In addition, imagoes of the PRHB have been sighted in other areas adjacent to Río Encantado (Morales and Estremera, Liga Ecológica Quebradillana, 2018, unpublished data, entire).

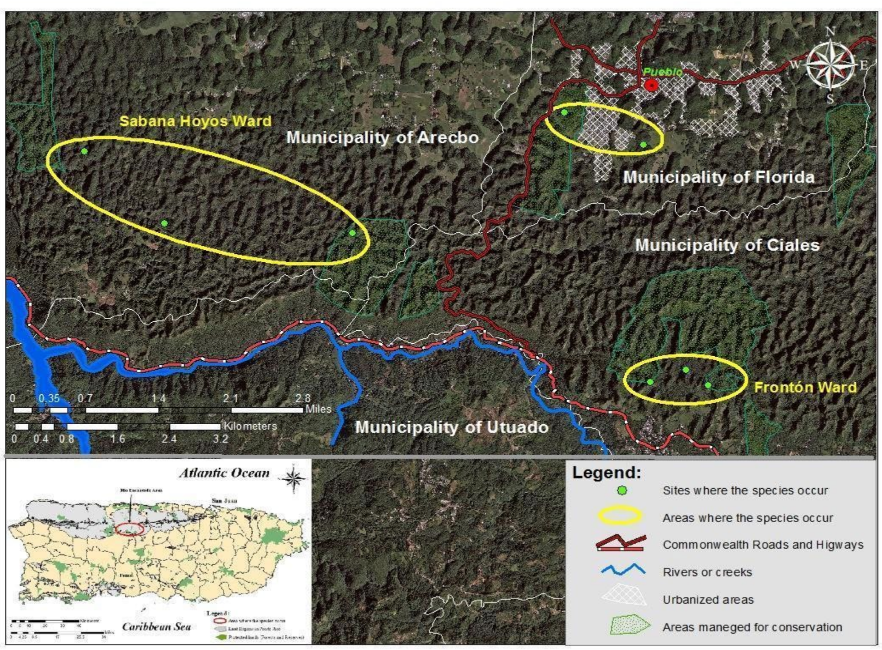


Figure 3-5. Map showing the distribution of the Puerto Rican harlequin butterfly in the Río Encantado area.

3.3.1.3 Río Abajo Commonwealth Forest Metapopulation

The Río Abajo Commonwealth Forest is located west of the Arecibo River, between the municipalities of Arecibo and Utuado. This forest is a public land managed for conservation and passive recreation by the Puerto Rico Department of Natural and Environmental Resources (PRDNER; historically Department of Natural Resources, DNR) since 1935 (DNR 1976), and is located approximately 29.9 km (20 mi) southeast of the Quebradillas' PRHB population, outside of the historical range of the species in the northern karst. Within the boundaries of the Río Abajo Commonwealth Forest, the species occurs in 3 locations, one adjacent to the west of State road PR-10 and another two close to Campamento Radley (Figure 3-6). All locations are in El Jobo Ward in Arecibo (Morale and Estremera 2018, Liga Ecológica Quebradillana, unpublished data, p. 7; J. Sustache, PRDNER, 2019, personal communication). In addition, sightings of imagoes of the species have been reported from other areas in Río Abajo Commonwealth Forest (J. Rios, Service, 2012, personal communication; Morales and Estremera, Liga Ecológica Quebradillana, unpublished data, 2018, O. Monsegur, Service, 2019, personal communication).

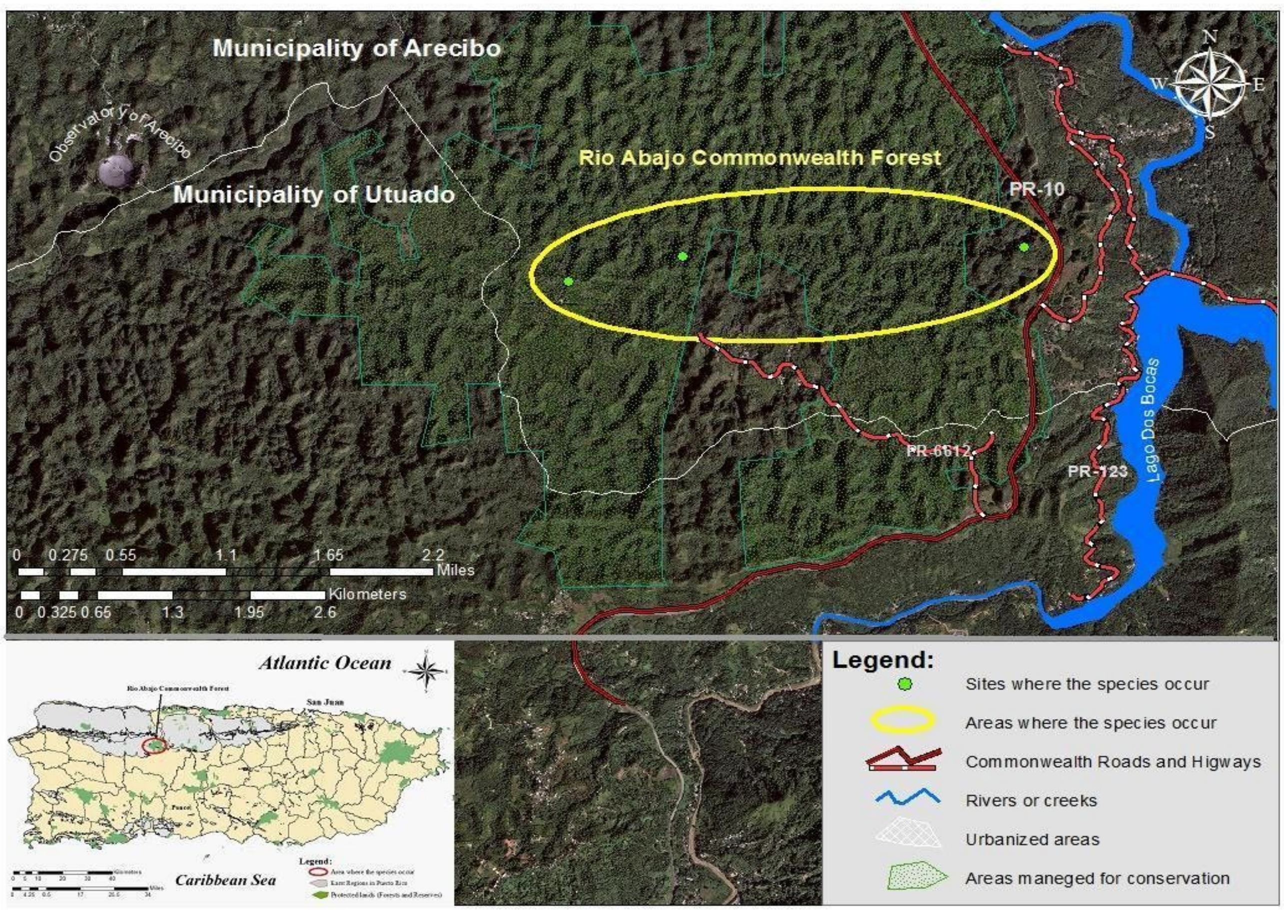


Figure 3-6. Map showing the distribution of the Puerto Rican harlequin butterfly at the Río Abajo Commonwealth Forest.

3.3.2 West-central Volcanic-serpentine Region

In the west-central volcanic-serpentine region, the PRHB occurs in the Maricao and Susúa Commonwealth Forests; both are public forests managed for conservation by the PRDNER.

3.3.2.1 Maricao Commonwealth Forest Metapopulation

The Maricao Commonwealth Forest is located in west-central Puerto Rico among the municipalities of Maricao, San Germán, Las Marias, Mayagüez and Sabana Grande, approximately 108.9 km (67.7 mi) west of San Juan (Pérez-Asso et al. 2009, p. 94). At the Maricao Commonwealth Forest, all life stages of the PRHB have been observed in 5 sites (Figure 3-7). Three of these sites are between the km 16.0 (mi 9.9) and km 16.8 (mi 10.4) markers of State road PR-120; one site is close to Campamento Buena Vista, a recreational area located adjacent to the Maricao Commonwealth Forest office; and another in a forested area near the National Parks Company camping area (Figure 3-7; Barber, 2018, pp. 22 and 29; C. Asencio, former professor Universidad Católica de Ponce, 2018, personal communication).

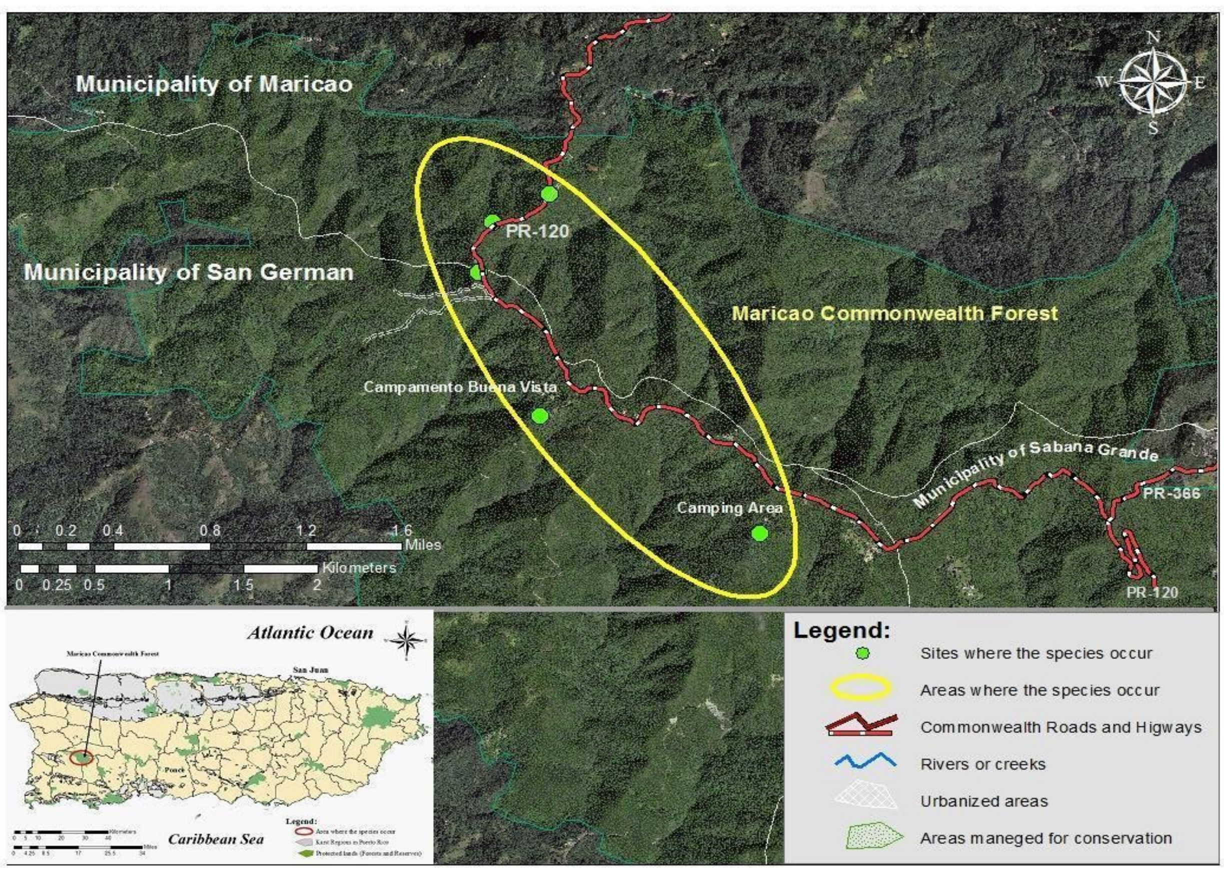


Figure 3-7. Map showing the distribution of the Puerto Rican harlequin butterfly at the Maricao Commonwealth Forest.

3.3.2.2 Susúa Commonwealth Forest Metapopulation

The Susúa Commonwealth Forest is located between the municipalities of Sabana Grande and Yauco, approximately 9.5 km (5.8 mi) southeast of the Maricao Commonwealth Forest. The PRHB has been documented in two (2) sites in the Susúa Commonwealth Forest (Figure 3-8; Barber 2016, p. 12). All life stages of the butterfly and the host plant have been observed on two (2) sections of the Eagle's trail, which is located in the southern section of the forest. Another site is located at Cuchilla Larga sector in the northern section of the forest.

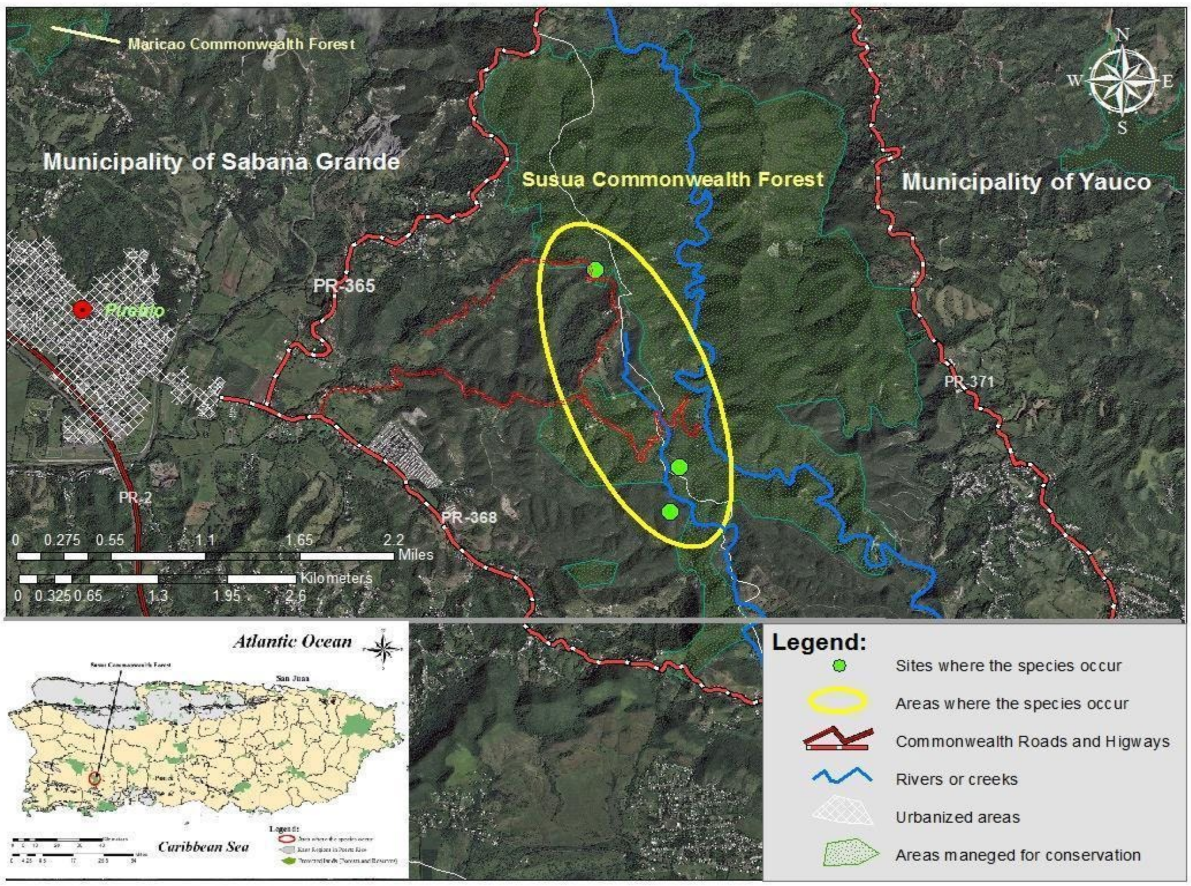


Figure 3-8. Map showing the distribution of the Puerto Rican harlequin butterfly at the Susua Commonwealth Forest.

3.4 Population Estimates

Since 2003, there have been several PRHB surveys, although survey methods and objectives have varied. Current (since 2003) population estimates are derived from anecdotal reports, species expert opinion, and number of individuals (imagoes or larvae) observed during single survey events. Thus, the estimated abundance of the species per population may vary according to the methodology implemented during the survey and the source of information. Assessing the overall population size and structure of the PRHB in the wild is considered a daunting task, particularly because of the apparent seasonality of the species (C. Pacheco, Service, 2019, personal observation). Although imago counts are considered a reliable method to estimate butterfly abundance, we cannot assume that all individuals are counted, because all species stages and instars are not likely to be detected during a single survey. Furthermore, the size and structure of the PRHB population is difficult to determine due to the lack of information regarding factors that can affect the population growth (e.g., amount of viable eggs per host plant, time of eclosion, mortality rate at each stage, among others). For example, the species may lay about 102 eggs per clutch, but subsequent counts after eclosions may yield a low number of imagoes (C. Pacheco, Service, 2019, personal observation). The lack of information on the population dynamics of the PRHB is a limiting factor in defining what constitutes a viable population. Consequently, estimating the overall abundance, population densities, size classes, or population trends for the butterfly is challenging because existing biological studies have not been designed specifically to determine these factors.

3.4.1 Population Estimates in the Northern Karst Region: IQC, Río Encantado, and Río Abajo Commonwealth Forest.

At IQC, the PRHB occurs in 13 areas (subpopulations), but recent surveys for the species have been conducted in only 7 of those 13 areas. In the municipality of Quebradillas, the surveyed areas include El Merendero, Puente Blanco and Puerto Hermina. By 2003, Carrión-Cabrera (2003, p. 60) observed 235 PRHB imagoes (mature adult stage) during the 12 months of surveys (2 sample days per month) on 0.34 ha (0.83 ac) in El Merendero and Puente Blanco. In addition, Carrión-Cabrera (2003, p. 61) reported that larval counts from April to July and from December to January resulted in between 100 and 200 larvae per survey day (2 man-hours of search efforts). It has been reported that larval abundance is lower during the rest of the year (i.e., February and September to November) (Carrion-Cabrera 2003, p.61). Later, the population was estimated to be 45 or fewer imagoes on any given day in the same area surveyed by Carrion-Cabrera in Quebradillas (Table 3; Biaggi-Caballero 2009, p. 4). The presence of more than one generation observed during the later survey confirms the species' multivoltine (producing several broods in a season) nature (Biaggi-Caballero 2009, p. 4). Based on the above information, by 2010, the PRHB population in the known sites in the municipality of Quebradillas was reported at around 50 imagoes and 100 larvae, for a density estimate of 132 imagoes and 294 larvae per ha (or 54 imago and 120 larva per ac) (76 FR 31282, May 31, 2011). The surveyed area was 2.68 ha (6.67 ac). The density is calculated as the number of individuals counted (abundance) divided by area in which they were observed.

In Isabela, over 200 imagoes of the species were observed during a one-day survey in December 2014, in an area of approximately 1.6 ha (3.9 ac) along the northern coastal cliff in Royal Isabela (J. Chabert, Fundación EL Pastillo, unpublished report 2015, p. 1), which equates to an estimate of 125 imagoes per ha (or 51 imagoes per ac).

More recently, Barber (2018, p. 1) surveyed the species after the Hurricane María in 6 sites (i.e., El Pastillo, El Tunel de Guajataca, Cara del Indio, El Merendero, Puente Blanco and Puerto Hermina) between the municipalities of Isabela and Quebradillas, and reported a maximum of 53 adults and a maximum of 1,381 larva in 2.68 ha (6.67 ac), or an estimated density of 20 imagoes and 515 larva per ha (or 8 imagoes and 207 larvae per acre) (Table 3-2).

The species abundance for the subpopulations in Río Encantado and Río Abajo Commonwealth Forest is currently unknown. The information available for these populations is based on sporadic sightings of the species (larva or imago), but not counts of individuals.

3.4.2 Population estimates in the West-central Volcanic-Serpentine Region: Maricao Commonwealth Forest and Susua Commonwealth Forest

In the Maricao Commonwealth Forest, the PRHB is known to occur in three (3) areas: along State road PR-120; at Campamento Buena Vista; and at the National Parks Company camping area. By 2011, the PRHB population in the Maricao Commonwealth Forest was estimated in no more than 20 imagoes and over 100 larvae (76 FR 31282, May 31, 2011). Recently, after Hurricane Maria, Barber (2019, p. 4) conducted surveys over 12 months (March 2018-March 2019) in two (2) sites: Los Pinos and La Cantera in Maricao and found a maximum of 21 adults and a maximum of 632 larva in 1.08 ha (2.67 ac), which equates to a density of 19 imagoes and 584 larvae per ha (or 8 imagoes and 236 larvae per ac). Throughout the years, the Maricao Commonwealth Forest manager, Edwin Avila, has observed an undetermined number of imagoes of the PRHB in different sections of the forest (E. Avila, PRDNER, 2016, personal communication).

The occurrence of the PRHB in the Susua Commonwealth Forest has been recently confirmed. Therefore, historical information about status and population estimates of the species is not available (Barber 2016, p. 15). Nonetheless, Wetsy Cordero, Manager of the Susúa Commonwealth Forest, has observed an undetermined number of imagoes of the species in different sections of this forest (W. Cordero, PRDNER, personal communication, 2016). Barber (2016, pp. 12-15) documented one PRHB imago and one larva along Eagle's trail and at the Cuchilla Larga sector, respectively. More recently, Barber (2019, p. 56) surveyed the species in these two sites and reported a maximum of 16 imagoes and a maximum of 83 larvae in 1.08 hectares (2.67 acre) during the 12 months of surveys, an abundance estimated of 15 imago and 77 larva per ha (or 6 imago and 31 larva per ac) (Table 3-2).

Aside from the information provided above, no scientific data regarding the abundance of the species or populations trends are available.

Table 3-2. Number of Puerto Rican harlequin butterflies observed per population.

Region of Puerto Rico	Metapopulation	Number of Individuals Observed	Surveyed Area	Source of Information
Northern Karst Region	Isabela, Quebradillas and Camuy (IQC)	45 or less adults (imago) / 10 to 100 larvae	0.34 ha (0.83 ac)	Carrión-Cabrera 2003, p. 34, Monzón-Carmona 2007, p. 44, Biaggi-Caballero 2010, p. 4
		53 adults (imago) / 1,381 larvae	2.68 ha (6.67 ac)	Barber 2019, p. 4
West-central Volcanic-Serpentine Region	Maricao Commonwealth Forest	12 adults (imago) / no data about larvae	Not determined (unknown)	Asencio 1984, entire
		No more than 5 imagoes / no more than 10 larva	Not determined (unknown)	Carrión-Cabrera 2003, p. 48, Pérez-Asso et al. 2009, p. 94
		21 adults (imago) / 631 larvae	1.08 ha (2.67 ac)	Barber 2019, p. 4

Table 3-2 continued.

Region of Puerto Rico	Metapopulation	Number of Individuals Observed	Surveyed Area	Source of Information
West-central Volcanic-Serpentine Region	Susúa Commonwealth Forest	Unknown (Not observed since 1980's)	Not determined (unknown)	Biaggi-Caballero 2010, p. 4
		16 adults (imago) / 83 larvae	1.08 ha (2.67 ac)	Barber 2019, p. 4
Southern Karst Region	Tallaboa Peñuelas	Unknown (Not observed since 1926)	Unknown	Biaggi-Caballero 2010, p. 4

3.5 Habitat Description Per Population

Habitat descriptions and species occurrence records at the subpopulation scale, including floral composition, the distribution of *O. spinosa,* and localities of PRHB eggs, larvae, or imagoes, are available for several sites and are provided in Appendix 3.

3.6 Population Needs

Resiliency refers to a species' ability to sustain populations through periods of both favorable and unfavorable environmental conditions and/or anthropogenic impacts. The PRHB needs robust populations (resiliency) to withstand environmental stochasticity (i.e., normal conditions, year-to-year variations in environmental conditions such as temperature, rainfall, drought seasons, etc.), periodic disturbances (e.g., fires, hurricanes, storms), and anthropogenic stressors (e.g., habitat modification, deforestation) (Redford et al. 2011, p. 40).

Certain habitat features influence the demographic attributes that determine PRHB population resiliency (Figure 3-9).

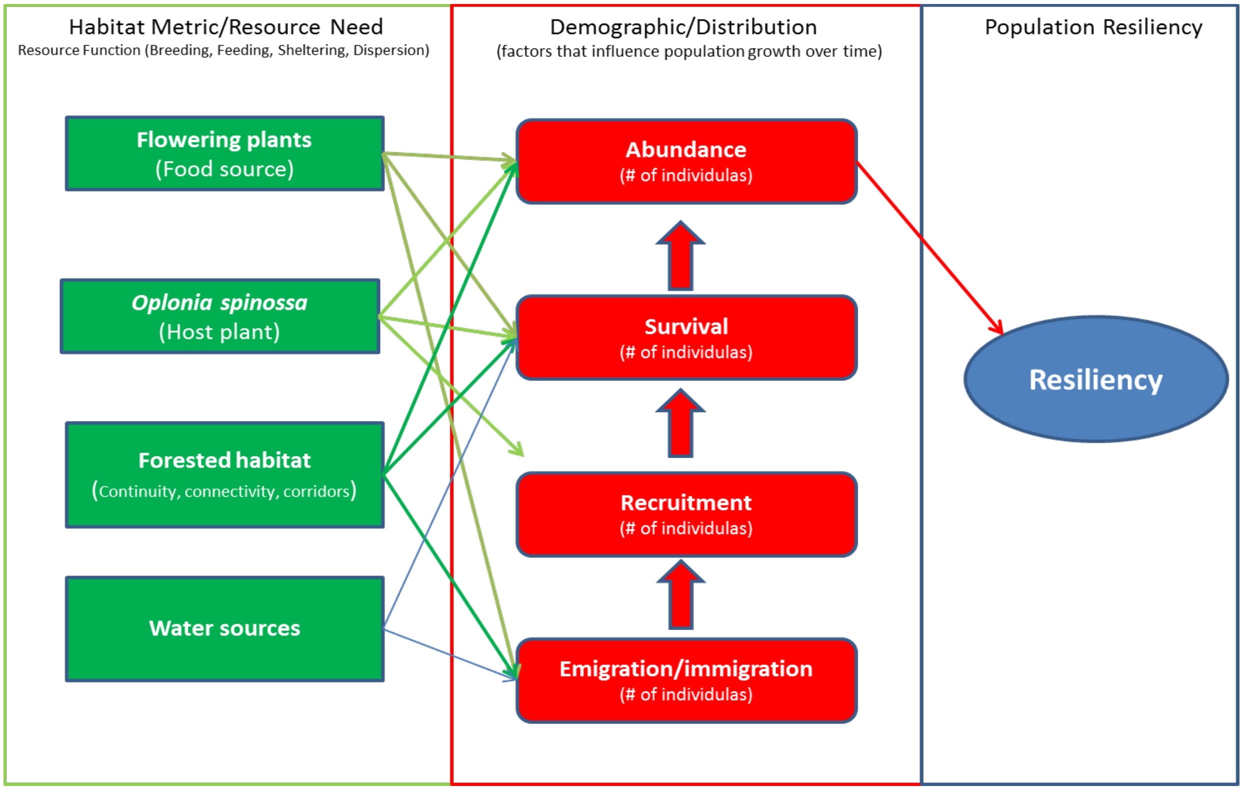


Figure 3-9. Basic conceptual model (or influence diagram) showing what resources may influence the resiliency of the Puerto Rican harlequin butterfly.

3.6.1 Interaction or Connectivity Among the Populations.

As discussed above (section 3.3), the PRHB is known from five (5) populations: three (3) in the northern karst region and two (2) in the west-central volcanic region. It is likely that these populations are not interacting, due to the distance between them and intervening habitat fragmentation resulting from past land use practices. However, each metapopulation is comprised of subpopulations distributed across the landscape. Based on the observed dispersal distance to mating sites (Monzón 2007, p. 42), these subpopulations are close enough (approximately 1 km [0.6 mi]) that adult individuals (imagoes) can interact. Persistence of the metapopulation depends not only on the fate of the individual subpopulations, but also on the influence of barriers to dispersal.

Healthy metapopulations rely on discrete high quality habitat patches, presumably those that are separated by less than 1 km (0.6 mi), and are embedded in a landscape matrix with few or only minor barriers to dispersal. We assume that all three factors, (1) short distances separating patches, (2) high quality habitat and (3) few or no dispersal barriers, are essential to ensure healthy PRHB metapopulation function, but we are uncertain of the relative importance of each factor.

3.6.2 Population Size, Demography, and Genetic diversity

The number of individuals comprising a metapopulation (population size) influences population viability through the processes of demographic, genetic, and environmental stochasticity. Metapopulation persistence requires robust demography, sufficient habitat, and some degree of genetic diversity.

Small and isolated populations frequently have low levels of genetic diversity, which reduces their capacity to respond to environmental change and can reduce population fitness via reductions in longevity, fecundity, offspring viability, and dispersal (Mattila et al. 2012, entire; Service 2018, p. 22). A positive relationship between genetic diversity and dispersal is mediated by proximate factors like flight metabolic rate, which can be diminished in small populations with low genetic diversity (Mattila et al. 2012, p. E2496; Hanski 2011, pp. 14401-14402; Rawlins and Lederhouse 1981, p. 387; Vandewoestijne et al. 2008, p. 8; Service 2018, p. 22). Low genetic diversity can also reduce longevity of butterflies and, thus, reproductive output (Vandewoestijne et al. 2008, p. 1). In addition, preservation of allelic diversity - the variety of alternate forms of genes - influences a population's ability to persist in the face of environmental change. High allelic diversity increases the likelihood that individuals will be adapted (i.e., possess genotypes that facilitate high survival) for varying environmental conditions. The interactions between genotype and temperature on flight metabolic rate and dispersal rate in Glanville fritillary butterflies (*Melitaea cinxia*), for example, strongly suggest that heterozygotes at a specific gene can reach higher levels of activity than homozygotes at low ambient and body temperatures but perform poorly at high temperatures (Niitepõld et al. 2009, p. 2230, Service 2018, p. 22). Having individuals with both gene combinations may ensure the population can persist through differing environmental conditions.

Genetic variation can be lost through genetic drift, which is driven by low effective population sizes (Furlan et al. 2012, p. 844). Thus, we believe that preserving the genetic diversity of the PRHB requires maintaining larger populations and connectivity among the populations, but data are not available to determine what constitutes a viable population size. In absence of a population viability analysis (PVA) to support a population target, we use the highest reported population index (amount of individuals counted on a determined time) as an indicator of population resiliency. Barber (2018, p. 1) reported densities of 20 imagoes and 515 larvae per hectare (or 8 imagoes and 207 larvae per acre) in Isabela, Quebradillas and Camuy; 19 imagoes and 584 larvae per hectare (or 8 imagoes and 236 larvae per acre) in the Maricao Commonwealth Forest; and 15 imagoes and 77 larvae per hectare (or 6 imagoes and 31 larvae per acre) in the Susúa Commonwealth Forest. Based on the previous information, we assume that higher resiliency subpopulations consist of at least 20 imagoes and 500 larvae per hectare (or 8 adults and 200 larvae per acre). However, the sex ratio of a natural PRHB population is unknown.

3.6.3 Health of the Populations

It is well known that butterflies are sensitive to environmental conditions, and experience swings in population numbers from year-to-year that vary among species according to life-history and other factors (Serrat et al 2015, p.207; Service 2018, p. 23). Species that are more sensitive to environmental conditions tend to fluctuate more drastically, and thus, require strong growth rate potential to recover in a short period of time. The PRHB should have a strong growth potential to recover when environmental or human-caused factors result in low survival or reproduction. Presently, the population growth to sustain a healthy population of the PRHB over time is unknown. Monitoring conducted in three (3) of the five (5) populations suggests that there are inter-annual fluctuations in the PRHB abundance and it may be lowest during February and October-November (Carrión-Cabrera 2003, p. 45; Barber 2019, p. 2). Nonetheless, there is no evidence that the species has significant year-to-year fluctuations in population size.

Population health is also affected by dispersal. Although evidence for a positive relationship between adult density and dispersal is not consistent among butterfly species (Konvicka et al. 2011, p. 98; Nowicki and Vrabec 2011, p. 663; Service 2018, p. 23), there is growing evidence that dispersal is positively related to genetic diversity and that genetic diversity is higher in large populations (Vandewoestijne et al. 2008, p. 5). Genetic drift is more likely to occur when populations are small and isolated. Dispersal is necessary for the PRHB to colonize or recolonize remnants of native forest harboring the host plant to facilitate gene flow and reduce the potential for genetic drift and inbreeding depression. Monzón (2007, p. 53) observed that the PRHB in Quebradillas has experimented local extinctions in some subpopulations, followed by re-colonization of un-occupied nearby patches harboring suitable habitat for the species. The author also found that imagoes used forested habitats as corridors between patches of suitable habitat.

Healthy PRHB metapopulations rely on discrete high quality habitat patches separated by less than one kilometer, and which are embedded in a landscape matrix with few barriers for dispersal of the species (Monzón 2007, p. 53, Morales and Estremera 2018, p. 1, Barber 2019, p. 1). All three factors are likely essential to ensure a healthy metapopulation function: short distances between patches; high quality habitat; and few or no dispersal barriers. However, we are uncertain of their relative importance. In a study of another butterfly species with "rather low dispersal ability" distance was the most important determinant of dispersion; habitat quality in recipient patches was second in importance, whereas matrix composition was of less importance (Kalarus and Nowicki 2015, p. 9; Service 2018, p. 23). As previously stated, the PRHB may not typically move greater than 1 km (0.6 mi) between habitat patches separated by structurally similar natural habitats, or through a mosaic of disturbed habitat including houses, roads and grass-dominated fields or pasture. Hence, habitat quality, indicated by factors including density of *O. spinosa,* amount and quality of adult food sources and low presence of predators, plays an important role in PRHB colonization success.

3.6.4 Habitat Considerations

In addition to population size, the capacity for PRHB populations to grow may be limited by the quantity and quality of the habitat, level of habitat disturbance, and the connectivity among habitat patches (Table 3-3). The minimum extent of habitat that is sufficient to support a healthy local population of this butterfly is unknown, but subpopulations are known to occur in patches of remnant native forests as small as one acre. Subpopulations in patches this small likely rely heavily on the existence of other subpopulations in nearby patches to ensure their long-term persistence.

The PRHB habitat consists of four general forest types already described above (i.e., subtropical moist forest on the northern coastal cliffs, subtropical moist forest on limestone-derived soil, subtropical wet forest on serpentine-derived soil, and subtropical dry/moist forest on serpentine-derived soil). Through this range of habitat types, the species occurs in patches of forest with canopy cover ranging from 50 to 85 percent, and an average canopy height of 6 m (20 ft). Forest connectivity among suitable patches and water sources, are also essential for the species. Historically, natural processes such as drought, hurricanes and storms have maintained a shifting matrix of suitable habitat (Lugo 2000, p. 244). However, anthropogenic disturbances (e.g., urban development, vegetation clearance, human induced fire, agricultural practice) also have been responsible in maintaining shifting matrix of suitable habitat and the essential features for the PRHB (Monzón 2007, p. 12). Moreover, given the short dispersal capability of the species, the frequency and intensity of these disturbances shifting in habitat may promote local extirpations of the PRHB (see Chapter 4- Factors Influencing the Species).

The PRHB occurs at elevations from 3 m (9 ft) to 867 m (2,845 ft) from sea level. However, only certain localities harbor the elements to sustain PRHB reproduction and development. In addition to *O. spinosa*, suitable sites must contain the right temperature and humidity levels (Perez-Asso et al 2009, p. 10). Average daily maximum temperatures where the species occurs range from 82 to 90ºF (28 to 32ºC), suggesting that the species' ecological niche has evolved within this range of upper thermal tolerance.

Oplonia spinosa coverage of more than 30 percent is an essential PRHB need. In 2014, Andrés Vélez (Graduate student from University of Puerto Rico, Mayagüez Campus) developed a habitat model to predict the possible distribution of the host plant *O. spinosa* in Puerto Rico (Vélez 2014, entire). This model was based on herbarium collections of the species. He found that *O. spinosa* may have a wide distribution in Puerto Rico, as well as the PRHB (Figure 3-10). Moreover, he identified three (3) hot spots where *O. spinosa* and the butterfly are predicted to occur.

Table 3-3. Parameters and requirements needed by the Puerto Rican harlequin butterfly at the population level to influence its resiliency, redundancy and representation.

Parameter	Requirements	Influence
Population size	Sufficiently large number of individuals to withstand unfavorable years and to avoid deleterious effects from genetic drift and inbreeding depression.	Resiliency Redundancy Representation
Habitat quality & quantity	Large patches (population-specific, but generally more than 1 acre) of native forest habitat with canopy cover from 50 to 85 percent, canopy height average of 20 feet with plant host covering more than 30 percent of the understory, and water source.	Resiliency
Habitat disturbance frequency and intensity	Low intensity and frequency of disturbance, and timing of disturbance does not occur during mating periods.	Resiliency Redundancy
Connectivity	Forested corridor between breeding sites. Suitable landscape matrix to allow movement between habitat patches (i.e., habitat patches < 1 km or 1000 m apart) on average and permeable land cover between patches	Resiliency Redundancy Representation

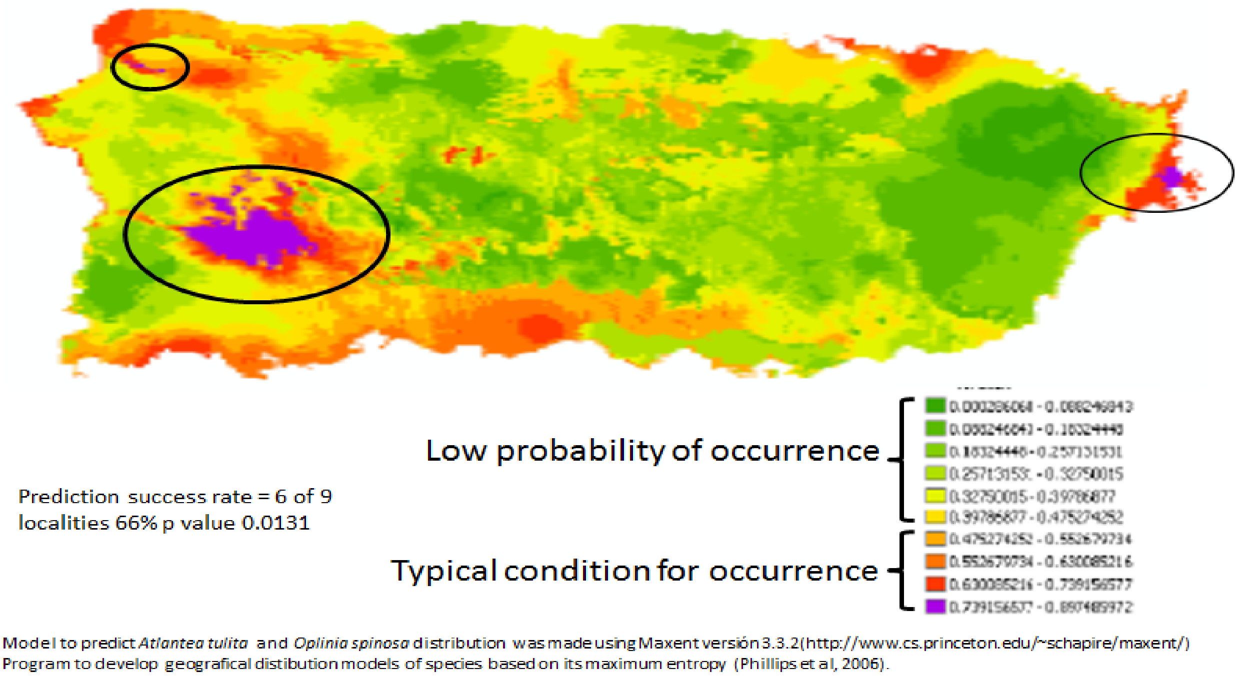


Figure 3-10. Map showing predicted areas with potential suitable habitat for *Atlantea tulita* and its host plant, *Oplonia spinosa* (Vélez 2014, entire).

3.7 Species Needs

The ecological requisites at the species level include having sufficient numbers, health, and distribution of populations to ensure it can withstand annual variation in its environment (resiliency), catastrophes (redundancy), and novel biological and physical changes in its environment (representation) (Table 3-4).

Table 3-4. Summary of the Puerto Rican Harlequin butterfly needs at the species level

3Rs	Requisites at Species-level	Details
Resiliency	Healthy populations distributed across environmental and habitat heterogeneous conditions	Environmental heterogeneity is having populations occupying areas with temperature and precipitation gradients; wet and dry habitats; and both north and south facing slopes.
Redundancy	Healthy populations distributed across geographical areas with low risks to catastrophic droughts and widespread pest control events	The intensity and duration of drought causing catastrophic losses is unknown,
Representation	Having healthy populations representing the breadth of adaptive diversity and maintaining evolutionary processes	Adaptive diversity is the variation in genetic and phenotypic traits that enable a species to adapt to novel changes. To ensure the breadth of adaptive diversity is preserved, we should maintain populations in their four native ecological settings (life zones) in Puerto Rico and conserve or promote connectivity among populations to ensure gene flow and minimize genetic drift.

Resiliency is the ability to sustain populations in the face of environmental variation and transient perturbations. The PRHB resiliency is a function of the number of healthy populations and the distribution of these populations across heterogeneous conditions. A healthy population is defined above under "Population-level Ecology." Maintaining populations across its range and across a diversity of environmental conditions helps guard against concurrent losses of populations by inducing asynchronous fluctuations among populations (Sutcliffe et al 1996, p.86). The environmental correlates most likely to influence the PRHB population dynamics include winter-spring temperatures (i.e., cooler temperature), summer-fall temperatures and precipitation (e.g., hot, dry summers; raining). The magnitude of influence these conditions pose depends upon habitat and landscape characteristics (e.g., forest cover, topography, soils, etc.). Generally speaking, with a greater degree of spatial heterogeneity there will be less synchrony among PRHB populations, thereby affording the species' greater resiliency to environmental disturbance. Additionally, resiliency also requires connectivity among populations for gene flow and demographic rescue. Connectivity between subpopulations (meta-populations) allows gene flow, and thus increases genetic health of a population.

Redundancy at Species-level reflects the ability of a species to withstand catastrophes (i.e., extraordinary events that would be expected to cause population extirpation), and is best achieved by having multiple, widely distributed populations of the PRHB relative to the spatial occurrence of catastrophic events.

As further explained in Chapters 4, Factors Influencing Viability of the Species, we identified drought, hurricanes, and fire as plausible catastrophic factors. Although the species' ability to withstand catastrophes can be influenced by its health (i.e., a demographically robust population is more likely to withstand drought conditions), survival is most strongly influenced by exposure to such events. Exposure is a function of both the number of populations (the more populations, the less likely all will be exposed contemporaneously and to the same intensity) and the distribution of populations (the more widely distributed, the less likely all will be exposed). Thus, generally speaking, the greater the number of populations and the more widely distributed, the more redundancy the PRHB possesses.

Representation at species-level reflects the ability of the species to adapt to novel changes in its environment. Measured by the breath of genetic or environmental diversity within and among populations, representation gauges the probability that the species is capable of adapting to environmental changes. For adaptation to occur, there must be variation upon which to act (Niitepõld et al. 2009, p. 2230; Lankau et al. 2011, p. 320; Service 2018, p. 29) and functional evolutionary drivers. By maintaining the sources of diversity across the species' range, as well as the processes that drive evolution (particularly gene flow and natural selection), responsiveness and adaptability of the PRHB over time are preserved.

Presently, the genetic diversity of the PRHB and its capabilities to adapt to environmental conditions is unknown. But, given the extremely limited geographic distribution and low number of individuals known of the PRHB, it is highly likely that its genetic variability presented in each population is very low. This would result in a loss of alleles by random genetic drift, which would limit the species' ability to respond to changes in the environment (Honnay and Jacquemyn 2007, p. 823).

Maintaining the adaptive capacity of a species also requires preserving the processes such as natural selection, gene flow, and genetic drift (Zackay 2007, p. 1; Crandall et al. 2000, p. 291) that allow for evolution to occur (Crandall et al. 2000, p. 290; Sgro et al 2011, p. 327). Natural selection is the process by which heritable traits can become more (selected for) or less (not selected for) common in a population by favoring those traits that enhance survival (Hendry et al. 2011, p. 169). To preserve natural selection as a functional evolutionary process, it is necessary to maintain populations across the breadth of biological and ecological conditions (i.e., historical latitudinal, longitudinal, and elevational gradients, as well as climatic gradients) to which the species may continue to adapt.

CHAPTER 4 –FACTORS INFLUENCING VIABILITY

In this Chapter we describe the most relevant factors that may negatively or positively influence the continued existence of the PRHB (Figure 4-1). Factors having a negative impact on the butterfly are referred to as risk factors or stressors; whereas factors having a beneficial effect are referred to as supportive factors. We refer to stressors and supportive factors collectively as "influences." We searched for information (published, unpublished literature, and species expert) to identify past and current negative and beneficial factors that have influenced the status of the PRHB across its historical and current range. Each factor (stressor or supportive) is considered in terms of scale, intensity, and duration, and the impacts it is having on the species and habitat across of its life history stages. Some factors may affect the species at all life stages or all individuals across the species' range, while others may affect a specific life stage. Additional factors acting on individuals of the species may not rise to the level of affecting the species or population(s).

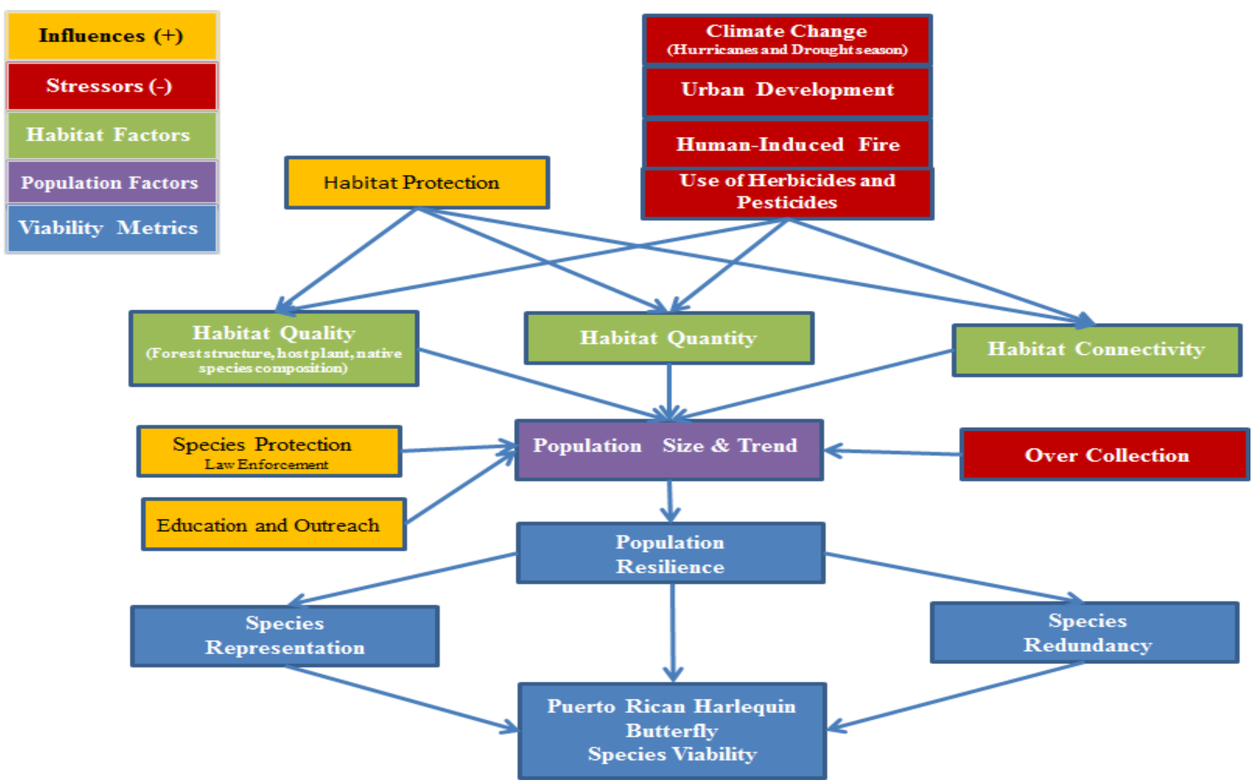


Figure 4-1. Key habitat factors, population factors, and supportive influences and stressors on viability used to assess resiliency, redundancy, and representation for the Puerto Rican harlequin butterfly.

Influences on the PRHB vary from location to location, but stressors include habitat loss and modification by development, clearing vegetation as maintenance activity, predators, human induced fires and changing climate, and poor enforcement of existing regulatory mechanisms are more wide-spread through its range. There is also evidence that the species has been collected for private entomology collections and un-authorized investigations. Positive influences on the PRHB have been habitat protection, habitat enhancement by reforestation and changes in habitat use.

4.1 Urban Development, Habitat Modification and Fragmentation

Habitat modification, fragmentation and loses caused by urban development and agricultural practices have been considered as the main factors influencing the decline of the PRHB, and pose continuing threats to the species' continued existence (6 FR 31282, May 31, 2011, p.31285; Barber 2019, p. 2). The species' small range may reflect a remnant population of a once widely distributed butterfly whose habitat was decimated by historic Puerto Rican land uses. Factors responsible for habitat loss, fragmentation, and degradation that we considered for this SSA analyses include conversion of native forest for agriculture or urbanizations; increase of highways and road (vehicle traffic); and land management regimes (vegetation clearance, grazing, haying). During European colonization, land was extensively modified for agricultural practices. A shift in the Puerto Rican economy from agricultural to industry led to land abandonment, and most of these lands where naturally reforested or converted for urban development. The consequences of the loss and fragmentation of natural habitat for the species may be detrimental because the PRHB seems to have low dispersal capabilities, a limited "patchy" distribution, and specialized ecological requirements, including laying eggs and feeding only on *O. spinosa* (6 FR 31282, May 31, 2011, p. 31286).

The PRHB faces significant threats from the existing and imminent destruction, modification, and curtailment of its habitat and geographic range in the municipalities of Isabela, Quebradillas, and Camuy. Historically, conversion of native forests into farms, pastures, or cropland, and in recent history, conversion of these to urban development, roads, recreational parks, and golf courses has been the most significant change in suitable habitat for the species in the IQC area. Most of the suitable habitat for the species, particularly in the municipality of Quebradillas, is currently fragmented by residential and tourist development. Dr. Stuart Ramos (University of Puerto Rico, Mayagüez Campus) reported that in 1997 one of the healthiest subpopulations showed a drastic decrease after the use of heavy equipment to clear vegetation in the Puente Blanco area in Quebradillas (Carrión-Cabrera 2003, p. 13). More recently, Barber (2016, p. 11) reported that in December 2016, in one of the areas where the species occurs in Puente Blanco, the vegetation was bulldozed, resulting in a decline of the *O. spinosa* and the PRHB. In areas where undeveloped land remains, the species' larval food plant is likely to be affected by existing vegetation management practices and agricultural practices that result in deforestation to increase grass lands for cattle grazing.

Although it is likely the historical and ongoing habitat degradation in the IQC area has reduced subpopulation connectivity and space for population growth, which reduces resiliency, persistence of the PRHB IQC population indicates the species can withstand, or at least recover from, some degree of disturbance. In Quebradillas, the PRHB has been observed in habitat previously disturbed by urban and agricultural development. Additionally, the IQC population inhabits a strip of forest that borders other patches of forest in various stages of succession that are fragmented by roads houses and agricultural lands.

PRHB habitat can be modified or lost by single land segregation for houses, large-scale residential and tourism projects, which are planned within and around its habitat in northern Puerto Rico. For instance, in the municipalities of Isabela and Quebradillas, occupied suitable habitat is within an area classified by both municipalities and the Puerto Rico Planning Board (PRPB) as a ‘‘Zone of Tourist Interest’’ (PRPB 2009, online data, at http://www.jp.gobierno.pr). A Zone of Tourist Interest (ZIT) is an area that has the potential to be developed to promote tourism due to its natural features and historic value. In 2010, 11 residential development projects were under evaluation around the species’ habitat, possibly affecting 29.4 ha (72.6 ac) in Quebradillas (Figure 4-2; PRPB 2010, online data). Reports from Ernesto Estremera (Ecological Alliance of Quebradilla, 2013, personal communication) indicate that over 20 residential and tourist development projects are proposed within the PRHB’s habitat. However, by 2018, only two new houses had been constructed and another is under construction at the Puente Blanco area (C. Pacheco, Service, 2018, personal observation). Most of these proposed projects may not be constructed in the near future due to the reduction in the economic activity in Puerto Rico; however, land owners have removed vegetation from the proposed projects sites, affecting the suitability of the habitat for the butterfly (C. Pacheco, Service, 2019, personal observation).

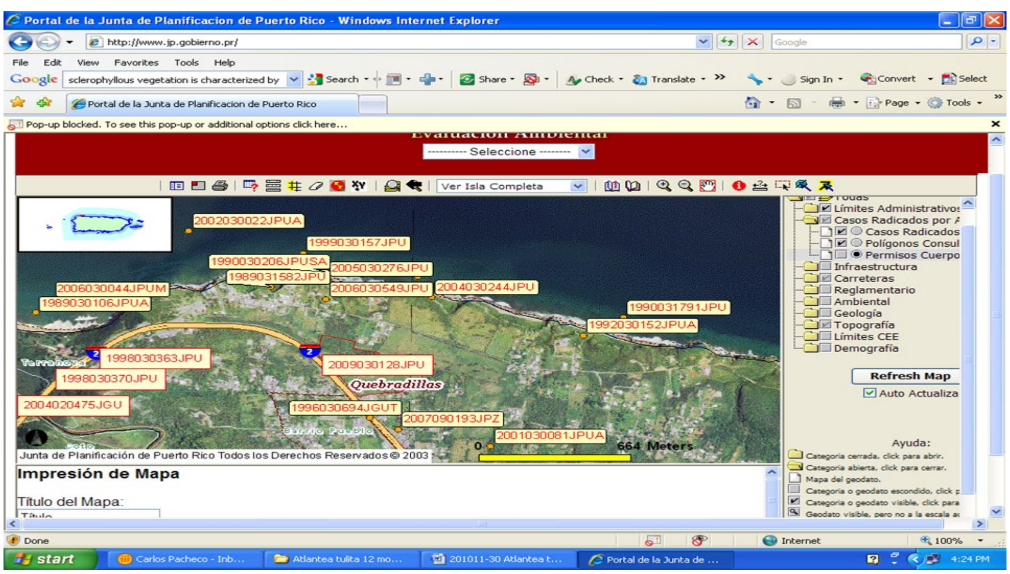


Figure 4-2. Photo showing the location of urban development project proposed for San José ward in Quebradillas. Screenshot taken on 8/2009.

The PRHB also occurs in areas managed for conservation (e.g., Río Abajo Commonwealth Forest, Maricao Commonwealth Forest, Susúa Commonwealth Forest, and Río Encantado Natural Area) or in areas where existing suitable habitat for the species may be protected by Commonwealth Laws and Regulations. However, some populations in or adjacent those areas could be subjected to habitat loss, modification, or fragmentation by urban development or by improvement of the infrastructure to support it. The number of houses around protected areas has continued increasing while human population in Puerto Rico is declining both around protected areas and island-wide (Castro-Prieto et al. 2017, p. 473). By 2016, a total of 32,300 new houses were constructed within 1 km of protected areas, while the human population declined by 28,868 within the same area. At the same time, 90 percent of protected areas showed increases in housing in the surrounding lands, 47 percent showed population declines, and 40 percent showed population increases, revealing strong spatial variations. Many of these new houses or the development of rural communities require construction of additional infrastructure (e.g., access roads, power energy service, water service, communication, among others), that increase their total effect on the surrounding habitats.

Urban development in or around PRHB habitat would directly and indirectly fragment and impact its habitat and would limit its population expansion through the area (Figure 4-3). Establishment of more residential and tourist development projects will result in an increase in access roads, vehicle traffic, and attendant road improvements in proximity to PRHB habitat. Construction of a new access road and improvement of the existing access road to existing or future rural communities may be considered as stressor that could directly (destruction of individuals or host plant) or indirectly (reducing forest habitat and food sources) reduce the populations of the PRHB and its habitat. Moreover, the proliferation of telecommunication towers has increased with the advent of cellular phone and related technologies. In Puerto Rico, towers for cellular communication, radio, television, military, and governmental purposes are a threat to plant species (such as *O. spinosa*) that happen to occur on top of mogotes (limestone hills) or mountaintops. While the towers themselves may not occupy a very large area, construction activities, access roads, and other facilities have a much wider impact, resulting in the elimination of potential habitat for the species. The biological effects of the existing roads and vehicle traffic on the species have not been studied. However, increasing vehicle traffic on roads within the essential habitat of a species that is a weak disperser may result in increased mortality due to collisions and, in some instances, can be catastrophic to species with low number of individuals per population (Glista 2007, p. 85).

The combination of habitat fragmentation and high road density may represent a challenge for the PRHB and could negatively impact the species and its habitat.

Figure 4-3. Photos showing some of the effects to Puerto Rican harlequin butterfly habitat caused by urban developments.

During the period from 1990 to 2010, the human population density of the northern karst region, which is about 75 percent of the entire karst region in area, increased from 1,225.97 inhabitants per square mile to 1,293.28 inhabitants per square mile (PRPB 2010, p. 45). The increase in population density of 5.2 percent during that period occurred despite the great migratory wave and loss of population during the decade of 2000-2010 for all of Puerto Rico, when hundreds of thousands of its residents moved to the metropolitan area and to various cities in the United States.

The proportion that represents the human population of the karst region with respect to entire Puerto Rico has increased over time. According to data from the 2010 Population and Housing Census, the karst region of Puerto Rico had a population of 2,763,178 inhabitants, representing 74.2 percent of the total population for Puerto Rico. However, Puerto Rico’s total population has been falling for nearly a decade, and the pace of decline has accelerated in recent years (Figures 4-4 and 4-5; Abel and Deitz 2014, p. 2). Since 2010, the population density of the karst region decreased from 1,326.36 inhabitants per square mile to 1,293.28 inhabitants per square mile. This represents a decrease of 2.6 percent, slightly higher than that of Puerto Rico overall, which was 2.2 percent. (PRAPEC, 2013, p. 18)

According to PRAPEC (2013, p. 19), the number of housing units in the karst region of Puerto Rico has increased in the last 40 years. According to the census of 1980, the total number of housing units was 762,485, and increased to 1,101,041 in the 2010 census, or a 44.4 percent increase. The total inventory of housing units of the Island has also increased in 64.7 percent in the last four censuses (1980-2010) representing a total of 1,636,946 homes for the year 2010. The percent increase of housing units in the karst region has fluctuated, with the largest increase in 1970 of 77.8 percent and the lowest increase in 2010 of 67.3 percent.

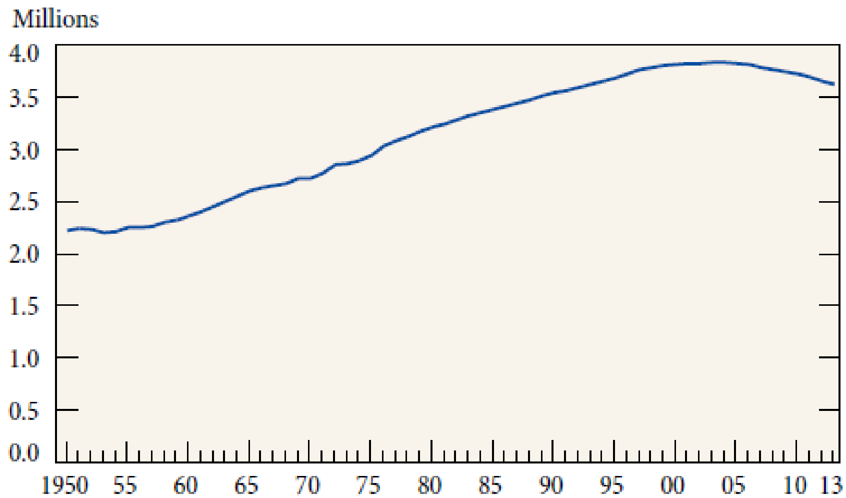


Figure 4-4. Graphic showing the Population in Puerto Rico from 1950 until 2013. Source: U.S. Census Bureau; Moody's Analytics. (Copied from Abel and Deitz 2014, p. 2)

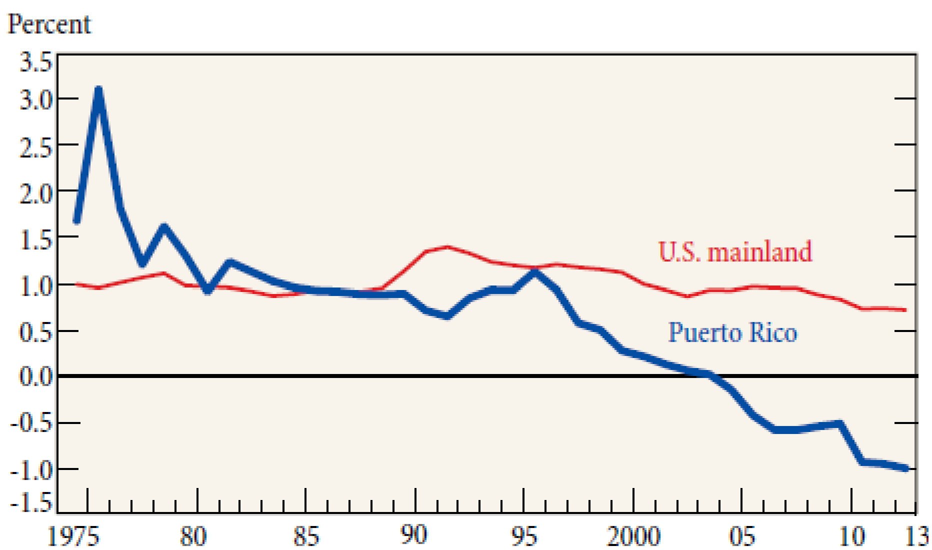


Figure 4-5. Graphic showing the Annual population Growth Rates from Puerto Rico and the U.S. Mainland. Source: U.S. Census Bureau; Moody's Analytics. Copied from Abel and Deitz 2014, p. 2.

4.2 Fire

Wildfires are a major ecological disturbance, affecting ecosystem functioning and species composition in forests around the world (Bond et al., 2005, p. 525; Brandeis and Woodall 2008, p. 557; Santiago-García et al. 2008, p. 604; Mateos et al. 2011, p. 1001). Fire is not a natural event in subtropical dry or moist forests in Puerto Rico (Robbins et al. 2008, p. 530), thus its effects on the PRHB habitat and host plant could be catastrophic. As the vegetation in the Caribbean is not adapted to fires, damage caused by fires to the ecosystems, particularly to plants species composition, might be irreversible (Santiago-García et al. 2008, p. 604, Brandeis and Woodall 2008, p. 557; Mendez-Tejada et al. 2015, p. 361). Fire can eliminate or modify the habitat of the PRHB either temporarily or permanently, and promote habitat fragmentation (C. Pacheco, Service, 2019, personal observation). A fire may also have a direct impact on the PRHB by killing imagoes, eggs, larvae, and chrysalis on the host plants. Furthermore, human-induced fires modify the landscape by promoting non-native trees and grasses, and by diminishing the seed bank of native species (Robbins et al. 2008, p. 528; Brandeis and Woodall 2008, p. 557). In some cases, fires may maintain extensive areas of young forest and grasslands, slowing the recovery of ecosystems and, therefore, impairing the delivery of ecosystem services (Brandeis and Woodall 2008, p. 557). For example, the nonnative grass *Megathyrsus maximus* is well adapted to fires and typically colonizes areas that were previously covered by native vegetation, and their presence increases the amount of fuel and the intensity of fires (Thaxton et al. 2012, p. 100). Furthermore, Mendez et al. (2015, p. 353) found that changes in climatological factors (such as precipitation, temperature, relative humidity and wind), combined may increase the threat of forest fires.

Human-induced fires are a current threat for the PRHB and its habitat in Quebradillas and Maricao (Biaggi-Caballero 2009, p. 5; Biaggi-Caballero 2010, p. 10, C. Pacheco, Service, 2019, personal observation). Wildfires resulting from natural or anthropogenic origin are growing in size and frequency across Puerto Rico (Brandeis and Woodall 2008, p. 558; C. Pacheco, Service, 2019, personal observation). Although wildfires may occur year-round, the majority of wildfires on the island of Puerto Rico occur primarily in the first three months of the year, corresponding to the dry season. (Figures 4-6 and 4-7; Mendez-Tejeda et al. 2015, p. 362). The Maricao Commonwealth Forest has been subjected to human-induced fires, potentially affecting the habitat used by the PRHB. The PRHB occurs on the driest section of this forest, near road PR-120. On February 25, 2005, a human-induced burned more than 400 acres with unknown effects on the PRHB population (Biaggi-Caballero 2010, p. 10). This fire likely had at least temporary effects on the butterfly's habitat, but we have no information regarding these effects and whether or not they were permanent. In Quebradillas, the species' habitat in Puente Blanco, where the most significant population occurs, is threatened by fires associated with clandestine garbage dumps on road PR-4485 (PRDNER, unpublished data, 2010, p. 23). Also, on March 3, 2019, a Service biologist visited the Cuchilla Larga site in the Susúa Commonwealth Forest. There he observed that an area of approximately 25 square meters of the habitat where the species occurs was burned in association with a clandestine garbage dump (C. Pacheco, Service, 2019, personal observation).

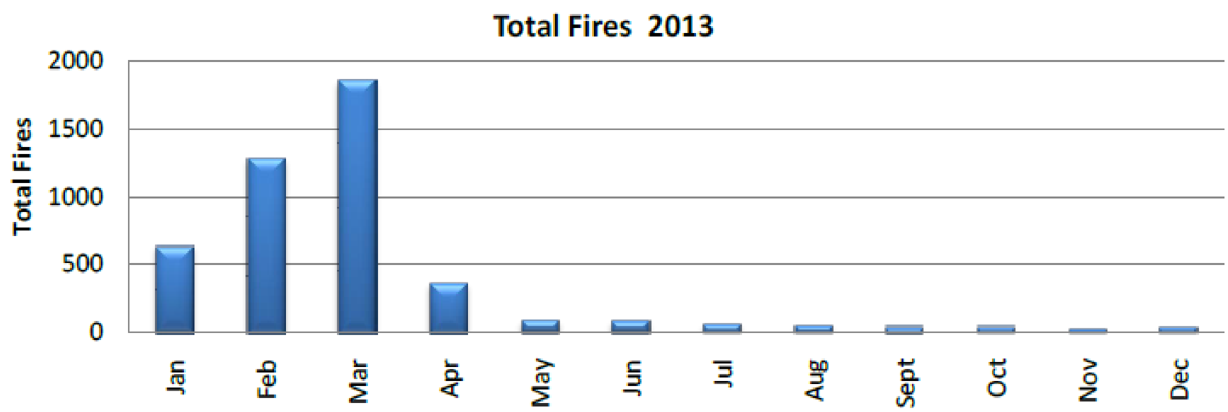


Figure 4-6. Wildfires per month that occurred in Puerto Rico during 2013. (Mendez-Tejeda et al 2015, p. 362).

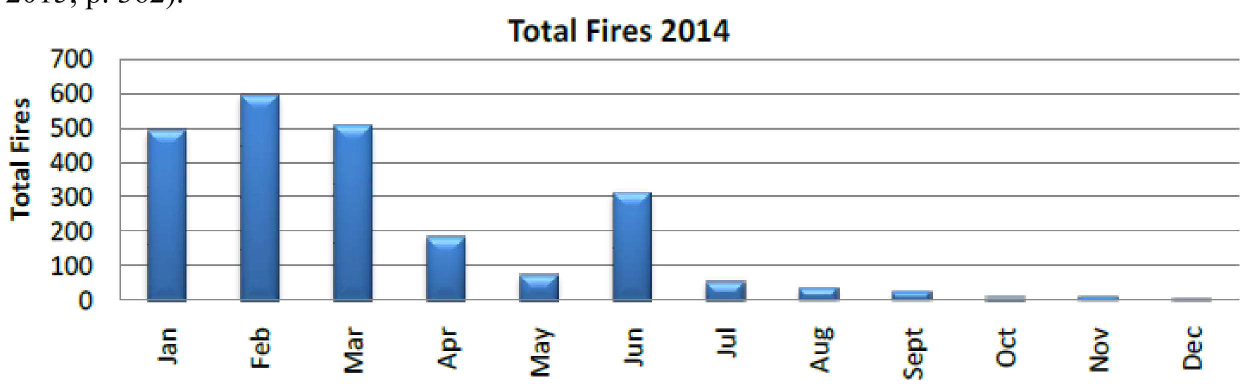


Figure 4-7. Wildfires per month that occurred in Puerto Rico during 2014. (Mendez-Tejeda et al 2015, p. 362).

4.3 Use of Pesticides, Herbicides, and other Mechanisms of vegetation control

Pesticide impacts to the PRHB are primarily influenced by the extent of the butterfly's exposure to pesticides throughout its range. Presently, the majority of Puerto Rican farmlands are dominated by industrial agriculture, a system largely influenced by chemically-intensive food production that depend of pesticides and chemical fertilizers that may have negative effects on the environment and consumer health (Santiago et al. 2016, p. 1). The purpose of pesticides and their primary use in agriculture is to protect crops from pest such as insects, fungi and weeds; therefore, their use has been considered as an essential part to maintaining industrial crops and to increase the level of global food production. Pesticides which include herbicides, insecticides, and fungicides are commonly used throughout the PRHB range on crop fields, along public roads, and on private properties to control plant and animal pests.

Puerto Rico also has a long history of using pesticides, mostly insecticides, for mosquito control in and around urban areas. Fumigation programs are implemented by the local government authorities at Terranova and San José wards in the municipality of Quebradillas to control mosquito-borne diseases (Biaggi-Caballero and López 2010, p. 9). The PRHB is found in both

Terranova and San José wards in areas surrounded by residences. No pesticide use guidelines have been developed for application in areas where the PRHB occurs (Biaggi-Caballero and López 2010, p. 9).

Herbicides are used by conservation agencies, public agencies, and private organizations to control vegetation in an array of areas. The use of herbicides is a current threat to the PRHB and its host plant, which is found on the edges of roads and open areas. Herbicide is frequently used to control woody vegetation and weeds along the access road to Puente Blanco (road PR-4485) and private properties, affecting an undetermined number of *O. spinosa* (C. Pacheco, Service, 2019, personal observation). If this practice is not carefully conducted, herbicides can indirectly impact PRHB populations by eliminating or reducing the host and food plants, particularly if it is applied during critical periods of the life cycle of the butterfly. Generally, herbicides are considered to have minimal effects on insects because the active ingredients target plants. However, the indirect effects of herbicides on the PRHB are not well known.

Vegetation management at El Merendero in Quebradillas may adversely affect the PRHB and its host plant (Figure 4-8). *Oplonia spinosa* grows on both sides of the existing hiking trails and around the picnic areas at El Merendero. Maintenance personnel frequently trim the new growth of this plant to remove vegetation from the trails and picnic areas, which affect the harlequin butterfly as it uses the newest vegetative branches of *O. spinosa* for laying its eggs and feeding during the larval stages (Biaggi-Caballero and Lopez 2010, p. 2). On April 12, 2012, maintenance staff of the municipality of Quebradillas cleared approximately 1 acre (0.4 ha) vegetative cover within the species habitat at El Merendero. Trimming the host plant and clearing the vegetation in these areas may result in mortality of the PRHB eggs and larvae. Further, the coastline of Isabela and Quebradillas is under pressure for urban and tourist development, only small remnants of coastal vegetation conserved in the steeper areas of the northern cliff still exist. In this area, landowners clear vegetative cover to the edge of the cliff in order to have a better view of the ocean (Biaggi-Caballero and López 2010, p. 3; Barber 2016, p. 11; C. Pacheco, Service, 2019, personal observation). Currently, no guidelines about vegetation management and clearing have been developed to avoid or minimize effects to the species and its host plant.

Removal of vegetation for agricultural practices or changing the natural landscape in urbanized areas, use of pesticides and herbicides may result in both short and long term adverse effects to the PRHB. Habitat removal and alteration may have direct impacts and result in eggs, larvae and/or adults mortality. The reproductive behavior of the PRHB, including the cryptic nature of the species' larvae, and the cryptic behavior of the imagoes are conditions that may contribute to these effects. Habitat removal and degradation may also alter spatial arrangement of possible territories or home ranges, may result in losing suitable breeding habitat in the future, would result in the creation of open corridors for predators, and degraded habitat is more attractive to invasive exotic plant species that may outcompete the *O. spinosa*.

Figure 4-8. Photos showing evidence of vegetation clearances within the Puerto Rican harlequin butterfly range.

4.4 Low Number of Individuals and Specialized Ecological Requirements

The PRHB is currently characterized by perennially low numbers of individuals (less than 100 imagoes observed per year). Little is known about the phenology, natural recruitment, demography and habitat requirements of the species. The low number of individuals per population and its spatial distribution suggest that the species has specialized ecological requirements. The apparently low reproductive rate (average lifetime number of offspring produced by a member of a population) of the PRHB and its specific ecological requirements (e.g., single host plant species) for completing its life cycle, are limiting factors for the species. These characteristics make the species less resilient and resistant to stressors that may impact existing populations. Moreover, Carrion-Cabrera 2003 (p.46) also found that the number of larvae decreased as the number of imagoes increased, suggesting that the population dynamics of the species may be synchronized with a yet undetermined environmental factor.

In the absence of knowledge on the natural recruitment capacity, survivorship at all life stages, and habitat requirements of this species, it is difficult to predict the recovery of the species after stochastics events such as hurricanes, human-induced fire, severe drought, among other. Effects of stochastic events can be exacerbated by the low number of individuals known through entire range of the PRHB. In fact, any of the PRHB populations can be easily extirpated by a stochastic event. However, it is noteworthy that surveys in IQC and Maricao six to 18 months after Hurricane Maria revealed that the PRHB persisted.

4.5 Genetic Variation

Given the extremely low known number of individuals of the PRHB, it is highly likely that its genetic variability is very low. In order to safeguard the remaining genetic diversity, the protection of known adult individuals should be considered as a high priority for the conservation of the species. No information about the genetic diversity or adaptive capacity of the PRHB to overcome stochastic events is available. However, it is well known that gene flow influences genetic diversity by introducing new alleles into a population, and hence, increasing the gene pool size (Crandall et al. 2000, p. 291; Honnay and Jacquemyn 2007, p. 823; Zackay 2007, p. 1). We surmise that imagoes can fly among subpopulations, keeping some genetic diversity within a given metapopulation. However, it is unlikely that genetic exchange among metapopulations occurs, due to the distance and landscape barriers (e.g. livestock pastures, urban areas) between them. Based on the above, we consider the possible lack of genetic variation as a stressor to the species.

4.6 Climate Change

Changes in climate can have a variety of direct and indirect impacts on PRHB and its host plant, and can exacerbate the effects of other stressors. Rather than assessing "climate change" as a single stressor in and of itself, we examined the potential consequences to the species and their habitats that arise from changes in environmental conditions associated with various aspects of the climate change. Vulnerability to climate change impacts is a function of sensitivity to those changes, exposure to those changes, and adaptive capacity (IPCC 2012, p. 5; USGCRP 2018, 20:821).

Puerto Rico has a tropical climate, with a mean annual precipitation ranging between 500 mm (19.6 in) and 4,400 mm (173.2 in), and a mean annual temperature between 19.4°C (66.9°F) and 29.7°C (85.5°F) (Castro-Prieto et al. 2016, p. 3). The Intergovernmental Panel on Climate Change (IPCC) concluded that evidence of warming of the climate system is unequivocal (IPCC 2007a, p. 30). More recently, the 2018 U.S. Global Change Research Program (USGCRP) reported that the impacts of climate change are already influencing the environment and more frequent and intense extreme weather and climate-related events, as well as changes in average climate conditions, are expected to continue to damage ecosystems. Numerous long-term climate changes have been observed, including changes in arctic temperatures and ice, and widespread changes in precipitation amounts, ocean salinity, wind patterns, and aspects of extreme weather, including droughts, heavy precipitation, heat waves, and the intensity of tropical cyclones (IPCC 2007b, p. 7). While continued change in climate is certain, the magnitude and rate of that change is unknown in many cases. Species that are dependent on specialized habitat types, that are limited in distribution or that have become restricted to the extreme periphery of their range will be most susceptible to the impacts of climate change. As previously mentioned, the PRHB is currently known from the northern karst region and the west-central volcanic-serpentine region of Puerto Rico, and apparently requires of specific habitat characteristics (e.g., temperature, humidity, among others), making the species susceptible to the effects of climate change.

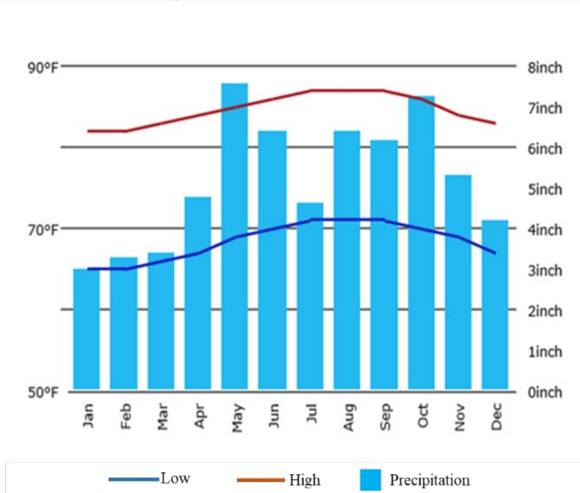
Studies conducted on other subfamilies of Nymphalidae (e.g., Danainae, Heliconiinae, Lycaenidae), reveal that temperature has a significant influence on imago and larval metabolism, growth rate and metamorphosis, and may affect seasonal colonization (local extinction followed by recolonization) and migrations (Rawlins and Lederhouse 1981, p. 403; Wong et al. 2015, p. 15; Koda and Nakamura 2010, p. 29; Franke et al. 2019, p. 1). Temperature in the range of the PRHB (Figure 4-9, 4-10, 4-11, and 4-12) is never constant on the scale of a day, week or month, and in many insects that are short-lived, the thermal conditions experienced during early life potentially have a large effect on their fitness (Wong et al. 2015, p. 15).

The continuously changing body temperatures associated with the environmental dependence also make unlikely the evolution of any acclimation mechanisms (Rawlins and Lederhouse 1996, p. 387). For example, the monarch caterpillar shows no indication of temperature acclimation (Wong et al. 2015, p. 16). Higher body temperatures within a 15-30ºC (59-86ºF) range not only hasten development but also affect other physiological factors influencing growth (Koda and Nakamura 2010, p. 29). Caterpillars of *Danaus chrysippus* have a larger final body size (5th instar) in colder parts of their distribution than those that live in warmer regions, which may explain the variation in the maximum weight of different ecotypes of insects with season and geographical area (Mathavan and Pandian, 1975, p. 63). As temperature increases toward 33ºC (91.4ºF), rates of ingestion, assimilation, and conversion to tissue all rise as metabolic maintenance costs fall in Palearctic *Danaus chrysippus* larvae (Mathavan and Pandian 1975, p. 63). At 37ºC (98.6ºF) these same larvae showed abrupt drops in conversion rates associated with falling ingestion rates and rising maintenance costs (Mathavan and Pandian 1975, p. 63). A similar pattern is expected in *D. plexippus* larvae, although at somewhat lower temperatures, since 100 percent mortality was recorded at 35.5ºC (95.9ºF) in the study. Since time spent feeding changed little during the day and rates of ingestion rise rapidly with body temperature, it follows that much less plant mass is consumed during the morning or evening, than during midday periods when body temperatures are elevated. Exposure to high temperature may also cause dehydration in butterflies, which is a serious threat to butterflies because of their large surface to volume ratio (Pometto 2014, p. 18). Day-fliers, such as the PRHB should have high need for water because they are active during the warmest time of the day, from 9:00 a.m. to 4:00 p.m. (C. Pacheco, Service, 2019, personal observations).

Climate for Isabela, Quebradillas and Camuy °C | °F

	Jan	Feb	Mar	Apr	May	Jun
Average high in °F:	82	82	83	84	85	86
Average low in °F:	65	65	66	67	69	70
Av. precipitation in inch:	2.99	3.27	3.39	4.8	7.56	6.38
Days with precipitation:	-	-	-	-	-	-
Hours of sunshine:	-	-	-	-	-	-

	Jul	Aug	Sep	Oct	Nov	Dec
Average high in °F:	87	87	87	86	84	83
Average low in °F:	71	71	71	70	69	67
Av. precipitation in inch:	4.65	6.38	6.18	7.24	5.31	4.21
Days with precipitation:	-	-	-	-	-	-
Hours of sunshine:	-	-	-	-	-	-



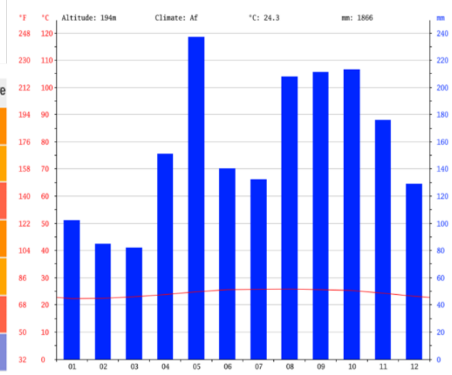
Annual high temperature:	84.7°F
Annual low temperature:	68.4°F
Average temperature:	76.55°F
Average annual precipitation - rainfall:	62.36 inch

https://usclimatedata.com/climate.php?location=USPR0046

Figure 4-9. Climate data for Isabela, Quebradillas and Camuy

Climate for Rio Abajo Commonwealth Forest and Rio Encantado Area, Puerto Rico

	Enero	Febrero	Marzo	Abril	Mayo	Junio	Julio	Agosto	Septiembre	Octubre	Noviembre	Diciembre
Temperatura media (°C)	22.3	22.4	23	23.8	24.8	25.6	25.7	25.8	25.6	25.3	24.3	23.2
Temperatura min. (°C)	17	16.8	17.3	18.2	19.4	20.2	20.5	20.5	20.4	20	19.2	18.2
Temperatura máx. (°C)	27.7	28	28.7	29.4	30.2	31	31	31.1	30.9	30.6	29.4	28.3
Temperatura media (°F)	72.1	72.3	73.4	74.8	76.6	78.1	78.3	78.4	78.1	77.5	75.7	73.8
Temperatura min. (°F)	62.6	62.2	63.1	64.8	66.9	68.4	68.9	68.9	68.7	68.0	66.6	64.8
Temperatura máx. (°F)	81.9	82.4	83.7	84.9	86.4	87.8	87.8	88.0	87.6	87.1	84.9	82.9
Precipitación (mm)	102	85	82	151	237	140	132	208	211	213	176	129



Adapted from: https://es.climate-data.org/america-del-norte/estados-unidos-de-america/puerto-rico/florida-766570/

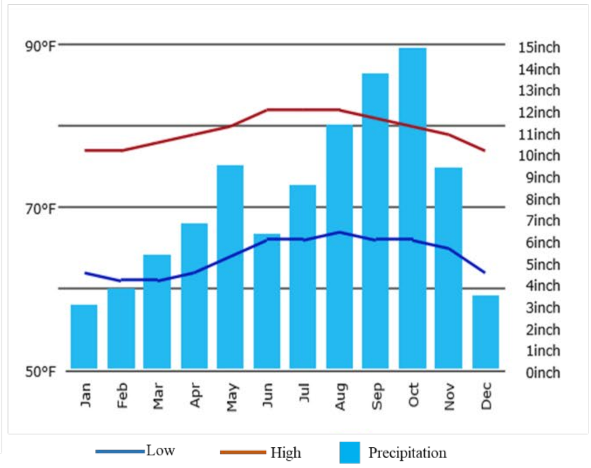
Figure 4-10. Climate data for the Rio Abajo Commonwealth Forest and Rio Encantado

Climate Maricao - °C | °F

	Jan	Feb	Mar	Apr	May	Jun
Average high in °F:	77	77	78	79	80	82
Average low in °F:	62	61	61	62	64	66
Av. precipitation in inch:	2.99	3.74	5.28	6.77	9.45	6.3
Days with precipitation:	-	-	-	-	-	-
Hours of sunshine:	-	-	-	-	-	-

	Jul	Aug	Sep	Oct	Nov	Dec
Average high in °F:	82	82	81	80	79	77
Average low in °F:	66	67	66	66	65	62
Av. precipitation in inch:	8.5	11.34	13.66	14.84	9.29	3.43
Days with precipitation:	-	-	-	-	-	-
Hours of sunshine:	-	-	-	-	-	-

Annual high temperature:	79.5°F
Annual low temperature:	64°F
Average temperature:	71.75°F
Average annual precipitation - rainfall:	95.59 inch
Days per year with precipitation - rainfall:	-
Annual hours of sunshine:	-
Av. annual snowfall:	-

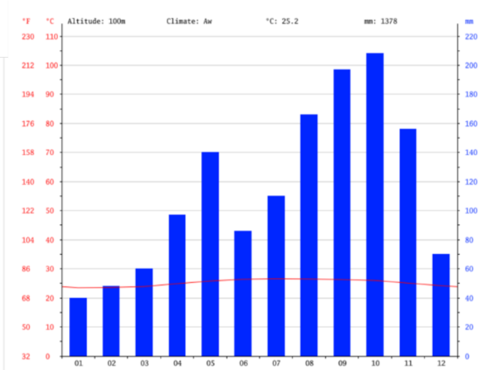


Adapted from website:
https://www.usclimatedata.com/climate.php?location=USPR0058

Figure 4-11. Climate data for Maricao Commonwealth Forest

Climate for Susua, Puerto Rico

	Enero	Febrero	Marzo	Abril	Mayo	Junio	Julio	Agosto	Septiembre	Octubre	Noviembre	Diciembre
Temperatura media (°C)	23.5	23.6	23.9	24.9	25.8	26.4	26.6	26.5	26.3	26	25.1	24.2
Temperatura min. (°C)	17.3	17.4	17.7	18.9	20.4	21	21	20.9	20.8	20.6	19.6	18.3
Temperatura máx. (°C)	29.7	29.8	30.2	30.9	31.3	31.9	32.2	32.2	31.9	31.4	30.7	30.1
Temperatura media (°F)	74.3	74.5	75.0	76.8	78.4	79.5	79.9	79.7	79.3	78.8	77.2	75.6
Temperatura min. (°F)	63.1	63.3	63.9	66.0	68.7	69.8	69.8	69.6	69.4	69.1	67.3	64.9
Temperatura máx. (°F)	85.5	85.6	86.4	87.6	88.3	89.4	90.0	90.0	89.4	88.5	87.3	86.2
Precipitación (mm)	40	48	60	97	140	86	110	166	197	208	156	70



Adapted from website https://es.climate-data.org/america-del-norte/estados-unidos-de-america/puerto-rico/sabana-grande-766574/

Figure 4-12. Climate data for Susúa Commonwealth Forest.

Puerto Rico is frequently in the path of hurricanes (Figure 4-13), and an expected effect of climate change is an increase in intensity of hurricanes and tropical storms, followed by an extended period of drought (IPCC 2012, p. 4). This change in climate can modify the microclimate, the plant species composition of the PRHB habitat, as well may affect the phenology of the *O. spinosa*. Hurricanes followed by extended periods of drought also may result in changes in soil conditions and microclimate, and may allow other plants (native or non-native, herbaceous or woody) adapted to drier conditions to become established (Lugo 2000, p. 243; Lugo 2008, p. 368). Invasive species (e.g. *Megathyrsus maximus)* may spread and colonize

O. spinosa habitat, promoting conditions for fires, and altering the microclimate and nutrient cycling of the habitat that is currently suitable for the PRHB.

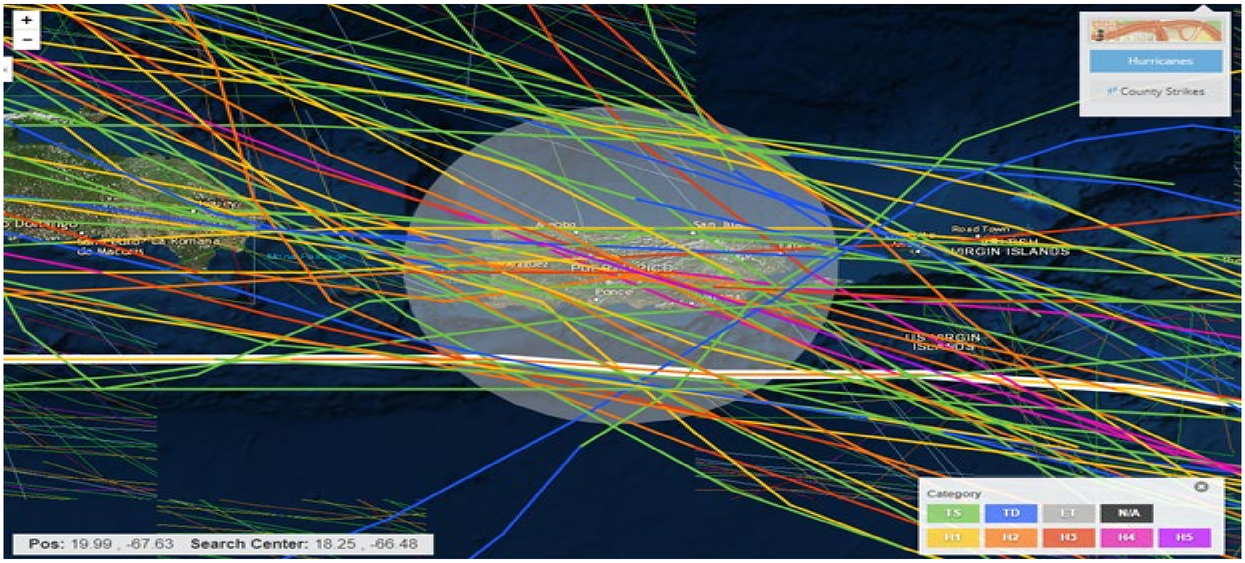


Figure 4-13. Historical hurricane tracks (late 1800s to current) in relation to Puerto Rico (NOAA 2018).

4.7 Over-collection

In addition to the threats mentioned above, we considered the possible effects on the PRHB of overutilization of the species for commercial, recreational, scientific or educational purposes. The PRHB is known for its rarity and restricted range, making the species attractive to collectors and scientists. Collection could be a significant threat to the species due to the few remaining populations, small population size, and the potential for collection to occur at any time due to the easy access by the public to PRHB populations in many locations. Because there is significant uncertainty regarding PRHB biology (i.e., abundance, distribution, habitat requirements, genetics and life history), any collection of imagoes, larvae or eggs without appropriate evaluation of its effects could adversely affect populations. Even limited collection from the remaining populations could have deleterious effects on reproductive and genetic viability of the species and could contribute to its extinction. An undetermined number of PRHB have been collected for scientific purposes and deposited in universities and private collections (J. Biaggi-Caballero 2011, personal communication.). However, at present few researchers are working with the PRHB, and its collection is regulated by the PRDNER. Although we consider collection to be a potential threat to this species, we do not have information indicating that the species is currently being collected for commercial, recreational, scientific or educational purposes.

4.8 Diseases and Predation

Due to the low number of individuals and known populations, disease and predation could certainly be threats to the PRHB. Biaggi-Caballero (2010, p. 8) and Chabert (2015, p. 3) suggested that spiders (i.e., *Misumenus bubulcus*, *Peucetia viridians*, *Argiope argentata* and *Nephila clavipes*) are a possible source of predation to the PRHB (Figure 4-14). They also mentioned lizards (i.e., *Anolis cristatellus* and *A. striatus*), and birds (i.e., *Tyrannus dominicensis, Dendroica adelaida adelaida*, and *Quiscalus brachypterus*) as possible predators. In fact, the sudden disappearance of larvae under study suggested depredation (Biaggi-Caballero 2010, p. 8). Moreover, we have found information suggesting that predation by anoles (*Anolis spp*.) and the spider *Argiope argentata* may affect the PRHB (Carrión-Cabrera 2003, p. 41).

Although the PRHB may face predation by spiders, ants, lizards, and birds, we are not aware of any data indicating predation is a significant threat to the species. Neither do we have information regarding any impacts from disease to the PRHB.

Figure 4-14. Potential predators of the Puerto Rican harlequin butterfly.

4.9 Regulatory Protection and Law Enforcement

The PRDNER designated the PRHB as Critically Endangered under Commonwealth Law No. 241 and Regulation 6766 (PRDNER 2004, p. 42; PRDNER 2010, unpublished data, p. 1). Article 2 of Regulation 6766 includes all prohibitions and states that the designation as "critically endangered" prohibits any person from taking the species; including to harm, possess, transport, destroy, import or export individuals, eggs, or juveniles without previous authorization from the Secretary of the PRDNER (PRDNER 2004, p. 28). Although, the PRDNER has not designated critical habitat for the species under Regulation 6766, Law No. 241 prohibits modification of any natural habitat without a permit from the PRDNER Secretary. The Service believes that Law No. 241 and Regulation 6766 provide adequate protection for the species. However, the lack of effectiveness of enforcement makes these policies inadequate for the protection of the habitat of the PPRHB, and particularly its host plant (Biaggi-Caballero 2010, p. 9). Biaggi-Caballero (2010, p. 9) stated that constant violation of the law occurs when the species' habitat is modified, destroyed, or fragmented for urban development and vegetation-clearing activities. The host plant is considered a common species associated with edges of forested lands and it is not directly protected by Law No. 241 or Regulation 6766. Previously, we discussed in more detail certain cases of lack of enforcement that have led to threats to the species and its habitat.

4.10 Habitat Conservation

The establishment of protected areas is the most frequently employed strategy to promote *in situ* biodiversity by conserving natural habitat, preventing its conversion to other uses, and reducing anthropogenic threats (Castro-Prieto et al. 2016, p. 1). The PRHB range includes a number of protected lands (e.g., Río Abajo Commonwealth Forest, Río Encantado Natural Protected Area and Maricao Commonwealth Forest) (Figures 4-15 and 4-16). Currently, over 64,683.4 ha (159,836.4 ac) of native forest along the northern karst belt are covered by the Karst Protection Law (Ley para la Protección y Conservación de la Fisiografía Cársica de Puerto Rico, Ley Núm. 292 de 21 de agosto de 1999) providing some regulatory mechanisms to protect that habitat. Conservation efforts have been directed towards land acquisition and conservation easements by government and non-government organizations (PRAPEC 2013, p. 19). In recent years, protection and management of the habitat that the PRHB share with other federally and state listed species (e.g., Puerto Rican parrot, Elfin woods warbler, among others) has become a high priority for the conservation of those species

For example, the Maricao Commonwealth Forest comprises 3,996.2 ha (9,874.8 ac) of public land managed for conservation (PA-CAT 2016, http://caribbeanlcc.org/interactive-map), that harbor habitat for the PRHB. Moreover, in 2000, PRDNER acquired through the USFS Forest Legacy Program a parcel of land of 107 ha (264.4 ac), locally known as “Finca Busigó”, adjacent to the Maricao Commonwealth Forest. This parcel is located approximately 1km from currently occupied PRHB habitat and is managed for conservation (PA-CAT 2016, http://caribbeanlcc.org/interactive-map).

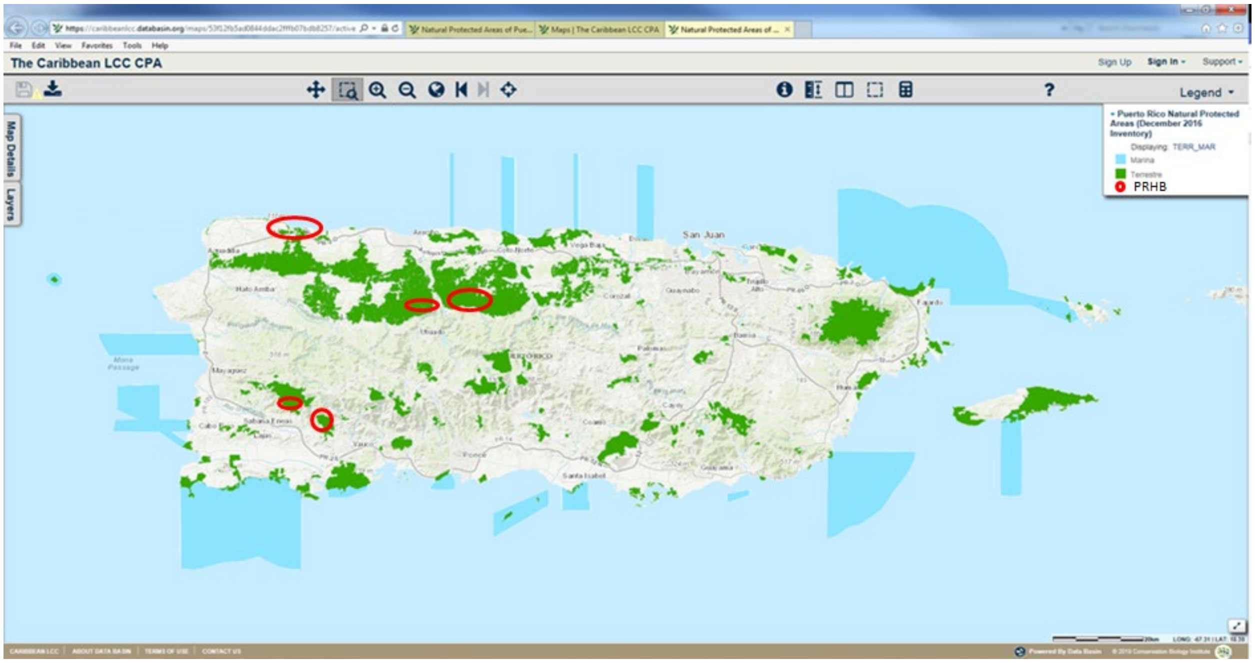


Figure 4-15. Current distribution of the Puerto Rican harlequin butterfly (red circles) in relation with the habitat under protective status (green polygons) in Puerto Rico (PA-CAT 2016, http://caribbeanlcc.org/interactive-map).

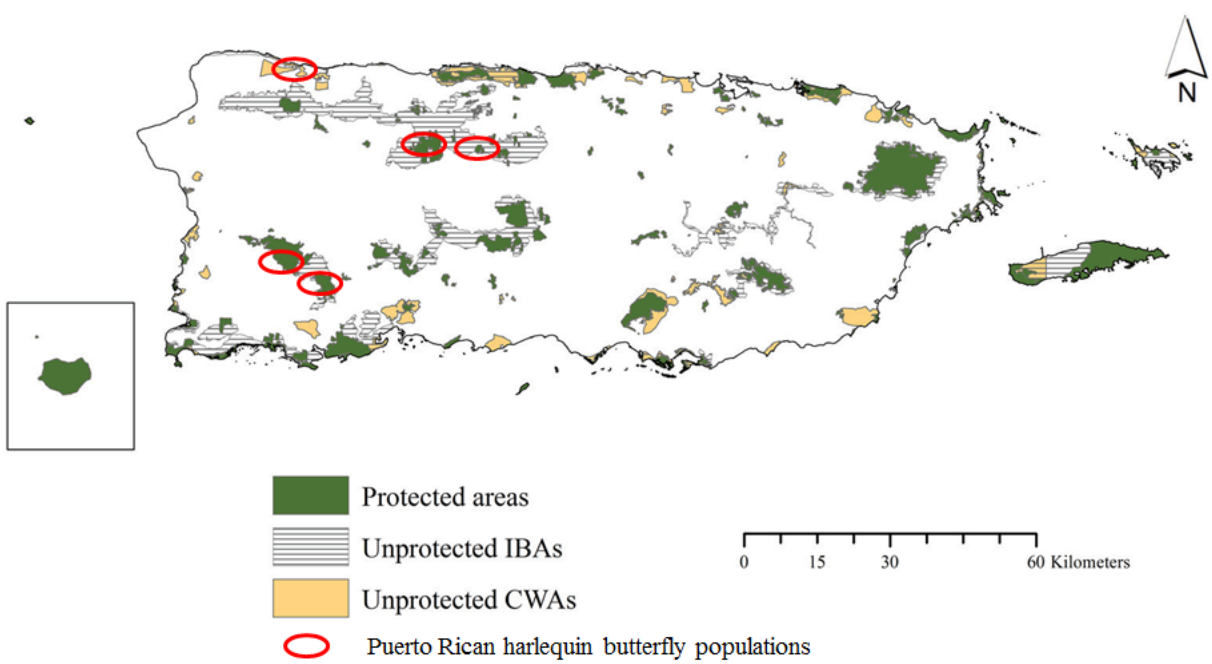


Figure 4-16. Map of Puerto Rico showing critical wildlife areas (CWAs) and important bird and biodiversity areas (IBAs) (Castro-Prieto et al. 2016, p. 11), red circles represent the locations of Puerto Rican harlequin butterfly populations.

4.11 Combined Effects of Stressors and Supportive Influences on Species Viability

The negative and positive effects of habitat alteration from several sources can combine to affect demographic attributes of populations, which, in turn, affects PRHB viability (Table 4-1).

Table 4-1. Habitat modification sources and effects that influence the demographic traits and viability of the Puerto Rican harlequin butterfly populations.

Sources	Effects	Demographic	Population
Commercial and urban development	(-) habitat loss, loss of connectivity, loss of food sources, loss of host plants, increase in vehicle traffic (road kills)	Survival, recruitment, emigration & immigration,	Decrease
Agricultural practices	(-) habitat loss, loss of connectivity (fragmentation), increase of open areas (deforestation/predation), loss of host plant, use of pesticides	Survival, recruitment, emigration & immigration	Decrease
Roads and Highways	(-) habitat loss, fragmentation, increase in vehicle traffic (road kills)	Survival, recruitment, emigration & immigration	Decrease
Hurricanes	(-) loss of food source, changes on forest structure, loss of host plant	Survival, recruitment, emigration & immigration	Decrease
Pest control (use of pesticides)	(-) decrease of suitability of the habitat, decrease of food sources,	Survival, recruitment, emigration & immigration	Decrease
Vegetation clearance (maintenance of green areas) Use of herbicides	(-)habitat loss, loss of connectivity (fragmentation), decrease food sources, decrease forest structure, change in microclimate/conditions, increase of open areas (deforestation/predation), loss of host plant	Survival, recruitment, emigration & immigration	Decrease
Predators	(-) decrease suitability of the habitat, loss of individuals	Survival, recruitment, emigration / immigration	Decrease

Table 4-1 continued.

	Sources	**Effects**	**Demographic**	**Population**
	Changes on the landscaping with non-native species (invasive species)	(-) outcompete with the host plant, changes on food sources	Survival, recruitment, emigration & immigration	Decrease
	Climate change (drought season vs rain season)	(-) changes in species composition, changes in phenology of food source species, changes on suitable habitat condition	Survival, recruitment, emigration & immigration	Decrease
	Wildfire (human induced fire)	(-) habitat loss, loss of connectivity (fragmentation), decrease food sources, changes in forest structure, change in microclimate/conditions, increase of open areas (deforestation/predation), loss of host plant, change species composition	Survival, recruitment, emigration / immigration	Decrease
	Reforestation	(+) increase in habitat; increase in shelters, increase in food source	Survival, Recruitment, Emigration / immigration	Increase
	Land acquisition/ conservation easements	(+) changes in land uses to allow natural reforestation, increase in habitat availability, reduction on stressors, increase in shelters, increase in food source	Survival, Recruitment, Emigration / immigration	Increase

CHAPTER 5 – CURRENT CONDITIONS

In this chapter, we consider the current condition of the Puerto Rican harlequin butterfly based on its distribution, abundance, and those factors currently influencing the viability of the species. We evaluate the needs of the species in terms of population resiliency and species' representation and redundancy (the 3Rs). Finally, we estimate the current condition of the species using habitat metrics to characterize the 3Rs.

5.1 Rangewide Conditions

Since the PRHB was added to the candidate species list (76 FR 31282, May 31, 2011), new information has become available about its distribution, abundance, habitat and factors that may influence its continued existence. The current distribution of the PRHB is depicted in Figure 3-2. As of 2019, this species has five (5) known populations consisting of 22 subpopulations persisting in four (4) life zones (or ecological settings) across three (3) ecological regions, at elevations from three (3) meters (9 ft) to 867 m (2,845 ft) from sea level. However, in any given year the maximum number of PRHB individuals recorded in its entire range is 90 butterflies and 2,096 caterpillars (Barber 2019, p. 1). Habitat loss and modification, followed by low number of individuals, have been identified as the most important stressors that may affect the continuing existence of this species.

5.2 Current Resiliency

In the SSA framework, resiliency is assessed at the population level, which is then scaled up to species redundancy and representation. Based on our knowledge on the PRHB, we believe that species population persistence is primarily influenced by the health of the subpopulations, connectivity among subpopulations, and risk due to stochastic events that may strongly affect the suitability of habitat on which the species depends. Moreover, the needs at individual level (suitable forested habitat with adequate food sources and the host plant, *O. spinosa*) must be met at a larger scale. Connectivity must be adequate not only for an individual's foraging needs, but to connect individual butterflies to a larger interbreeding population, influencing the probability of subpopulation persistence through both rescue effect and genetic health. Unfortunately, we are unable to reliably quantify the causal relationship between the degree of connectivity and subpopulation persistence. Similarly, we are unable to determine a reliable frequency estimate for stochastic events that may result in loses of individuals or habitat for the species. On the other hand, PRHB populations can persist in co-occurrence with predators like birds, spiders and ants, although the most resilient populations occur where these predators are few or absent.

Resiliency scores (Table 5-1) were generated by combining scores for four (4) habitat metrics (Protection/Development Risk, Connectivity/Habitat Fragmentation, Risk of Vegetation clearing/Use of Pesticides, and Susceptibility to Stochastic Events (e.g., human-induced fires, severe drought, hurricanes, among others) and one (1) population metric (population size or trend). Habitat metrics and the population metric were equally weighted. Each habitat metric was given one (1) point each for a total of 4 points. The population metric was weighted four (4) time higher than the each habitat metric (1) because it is a direct measure. As such, the population metric totals 4 points. Habitat metrics are still important, but do not reflect abundance and therefore do not have more weight than the population metric. Each habitat metric was assigned a score of 1, 2, or 3, and for the population metric was assigned a score of 4, 8, or 12, as described below in Table 5-1.

Table 5-1. Description of how habitat and population factors were scored to determine the Puerto Rican harlequin butterfly resiliency.

H Score	Habitat Metrics Influencing Viability				Population Metrics	P Score
	Habitat Protection	Connectivity/Habitat Fragmentation	Vegetation Clearing/Use of Pesticides	Susceptibility to Stochastic Events	Population size/Trends	
1 point each; 4 points total	Most habitat not protected, at risk of being developed (<34 percent protected)	Isolated subpopulations located at a distance of more than 1 km from the next one; habitat between populations or subpopulations highly disturbed (low connectivity)	Subpopulations located in areas subjected to vegetation clearing (including the use of herbicides) and use of pesticides (mosquito control and agricultural practice)	Subpopulations located in areas more vulnerable to stochastic events (e.g., fire, severe drought, hurricanes, among others)	Relatively low population size (0 to 5 imago and less than 100 larvae per ha) or high degree of uncertainty in population size/trends	4
2 points each; 8 points total	Some habitat protected, with some at risk of being developed (34-66 percent protected)	Subpopulations located within 1 km range; habitat between population moderately disturbed (some forested corridors)	Subpopulations located in areas rarely occur vegetation clearing (including the use of herbicides) or use of pesticides (mosquito control and agricultural practice)	Subpopulations located in areas with moderate vulnerability to stochastic events (e.g., fire, severe drought, hurricanes, among others)	Relatively moderate population size (6 to 20 imagoes and 100 to 500 larvae per ha)	8
3 points each; 12 points total	Most habitat protected (>66 percent)	Subpopulations located within 1 km range; un-disturbed habitat between populations (forested corridors)	Subpopulations located in areas where vegetation clearing (include the use of herbicides) or use of pesticides (mosquito control and agricultural practices) is not expected.	Subpopulations located in areas with lower vulnerability to stochastic events (e.g., fire, severe drought, hurricanes, among others).	Relatively high population size (more than 20 imagoes and more than 500 larvae per ha) and/or growth.	12

The score for each population across all metrics were summed, and final population resiliency categories were assigned as follows:

Low Resiliency:	<11
Moderately Low Resiliency:	11 to 13
Moderate Resiliency:	14 to 18
Moderately High Resiliency:	19 to 21
High Resiliency:	> 21

5.2.1 Current Resiliency of the Isabela, Quebradillas and Camuy (IQC) Population

The PRHB currently occurs in a 400 ha (988 ac) strip of forested habitat on a coastal cliff that extends along the municipalities of Isabela, Quebradillas, and Camuy. Currently, this strip of habitat is bounded on the east by the community La Yeguada and Membrillo in Camuy; on the west by the community Villa Pesquera and Pueblo in Isabela; on the north by the Atlantic Ocean; and on the south by state road PR-2, the Royal Isabela Golf Course, and a mosaic of forested patches that vary in size and stages of vegetation succession (Figure 3-1). These forested patches are fragmented by roads, houses and agricultural lands. Forest structure varies in the IQC. At the cliff edges, the vegetation is constantly swept by trade winds from the ocean. As a result of these winds, the trees are stunted and mostly sclerophyllous, and the forest is very thick, making it in most cases impenetrable. Moving from the cliff edge to the south, the canopy coverage increases from zero (0) percent up to 70 percent, and mean tree height up to around 6 m (20 ft) (Barber 2018, p. 14; Vargas 2019, p. 3). Presently, some habitat damage from Hurricane Maria is evident. The habitat appears to be recovering well (Carlos Pacheco, USFWS, 2019, personal observation.), although canopy height and density are significantly reduced from pre-hurricane levels.

Within IQC, the PRHB is regularly observed in 13 areas. Recently, five (5) of the 13 areas of occurrence (subpopulations) were monitored. The areas surveyed were El Pastillo, Cara del Indio and Tunel de Guajataca in Isabela; and El Merendero, Puente Blanco, and Puerto Hermina in Quebradillas. According to the results of the most recent surveys conducted after Hurricane Maria, the maximum total individuals counted in 2018 was 53 imagoes and 1,381 larvae in an area of 2.7 ha (6.7 ac) (Barber 2018, p. 1). Based on this information, the PRHB population has demonstrated resiliency to a severe hurricane, although it is too early to assess longer-term effects.

The PRHB occurs on both private and publicly owned lands, where in many places the species occurs close to urban development or lands used for agriculture. This is the case of the population located among the municipalities of Isabela, Quebradillas and Camuy (Figure 3-4), where the sites of occurrence are adjacent to areas already developed or undergoing development (Figure 4-3). In this population, three (3) of the 13 patches (subpopulations) fall within areas that may provide some protection, as they are in public or private lands managed for conservation, recreation, or as scenic areas. These three patches include El Merendero, El Pastillo, and Royal Isabela (Monzón-Carmona 2007, p. 84; Chabert 2015, p. 1). The other 10 patches are in Isabela and Camuy, and are in private lands subject to development.

Habitat occupied by the IQC population is largely unprotected and is at risk of being developed **(1)**. The IQC subpopulations are within 1 km of each other or forested habitat **(2)**. These subpopulations are located in areas subject to vegetation clearing (including the use of herbicides) and use of pesticides (mosquito control and agricultural practice) **(1)**. Additionally the subpopulations are located in areas moderately buffered from stochastic events (e.g., fire, severe drought, hurricanes, among others) **(2)**, and population size is high **(12)**. Therefore, the IQC population of the PRHB is considered to have **moderate resiliency (18)**.

5.2.2 Current Resiliency of the Rio Abajo Commonwealth Forest Population

The Río Abajo Commonwealth Forest is public land managed for conservation and passive recreation by the Commonwealth of Puerto Rico since 1935. In this forest, the species occurs in three (3) locations: one is adjacent to the west of PR road 10, and the other two are close to Campamento Radley. All locations are in El Jobo Ward in the Municipality of Arecibo. These sites are located within 1 km of each other. In addition, sightings of imagoes of the PRHB are frequently reported from other areas in Rio Abajo Commonwealth Forest.

The habitat in Río Abajo Commonwealth Forest is protected under Laws No. 133-1975 (12 L.P.R.A., Sec 191), known as *Ley de Bosques de Puerto Rico* (Puerto Rico Forests' Law), as amended in 2000. Section 8 (A) of this law prohibits cutting, killing, destroying, uprooting, extracting, or in any way hurting any tree or vegetation within a Commonwealth forest without authorization of the PRDNER Secretary (12 L.P.R.A. sec. 191f). The PRDNER also identified the Río Abajo Commonwealth Forest as a Critical Wildlife Area (CWA). The CWA designation constitutes a special recognition by the Commonwealth with the purpose of providing information to Commonwealth and Federal agencies about the conservation needs of these areas, and to assist permitting agencies in precluding adverse impacts as a result of permit approvals or endorsements (PRDNER 2005, pp. 247-350).

In the Río Abajo Commonwealth Forest there is substantial habitat protection **(3);** populations or subpopulations are within 1 km of each other and the habitat between populations or subpopulations is undisturbed forested corridors **(3)**; the populations or subpopulations are located in areas where vegetation clearing (including the use of herbicides) or use of pesticides (for mosquito control and agricultural practice) is not expected **(3)**; the populations or subpopulations are located in areas buffered from stochastic events (e.g., fire, severe drought, hurricanes, among others) **(2)**; and population size is low with a high degree of uncertainty in size and trends **(4)**. Therefore, the population of the PRHB in Río Abajo Commonwealth Forest is considered to have **moderate resiliency (15)**.

5.2.3 Current Resiliency of the Rio Encantado Population

The Río Encantado area comprises over 6,474.9 hectares (16,000 acres) considered by federal and Commonwealth conservation agencies as undisturbed wilderness, holding the largest tract of continuous forest cover in all Puerto Rico (www.paralanaturaleza.org/en/rio-encantado-eng). By 2010, Para La Naturaleza acquired approximately 809.3 hectares (2,000 acres) of this land for protection and conservation, and efforts to continue acquiring land are ongoing. Presently, the

PRHB is known to occur in three (3) areas scattered through the Río Encantado area. Within these areas, all life stages of the species and the host plant have been observed in eight (8) subpopulations. In addition, imagoes of the PRHB have been sighted in other areas adjacent to Río Encantado.

Although the Río Encantado area is covered by the Karst Protection Law (Ley para la Protección y Conservación de la Fisiografía Cársica de Puerto Rico, Ley Núm. 292 de 21 de agosto de 1999), private properties within this natural area its surroundings have experienced an increase of rural developments. According to the Karst Water Institute, the karst region of Puerto Rico is one of the 10 most endangered karst ecosystems in the world, and each year, up to 1 square kilometer of limestone rock is lost due to gravel pit extraction (https://www.paralanaturaleza.org/en/rio-ecantado-eng).

In the Río Encantado area there is substantial habitat protection, although there are some private lands and rural developments **(2)**; the populations or subpopulations are located within 1 km of each other, and the habitat between populations or subpopulations is undisturbed forest **(3)**, the populations or subpopulations are located in areas where vegetation clearing (including the use of herbicides) or use of pesticides (for mosquito control and agricultural practice) is not expected **(3)**; the populations or subpopulations are located in areas with low susceptibility to, or are buffered from, stochastic events (e.g., fire, severe drought, hurricanes, among others) **(2)**, and population size is low, with a high degree of uncertainty population trends **(4)**. Therefore, the population of the PRHB in the Río Encantado area is considered to have **moderate resiliency (14)**.

5.2.4 Current Resiliency of the Maricao Commonwealth Forest Population

The Maricao Commonwealth Forest is a public land administered by the PRDNER for conservation. Construction of power and communication structures is one of the threats to habitat in Maricao. The habitats on which the PRHB depends in the Maricao Commonwealth Forest are protected under the *Ley de Bosques de Puerto Rico* (Puerto Rico Forests' Law), as amended in 2000. The PRDNER also identified the Maricao Commonwealth Forest as a Critical Wildlife Area.

In the Maricao Commonwealth Forest habitat protection is substantial **(3)**; the populations or subpopulations are located within 1 km of each other, and the habitat between populations or subpopulations is moderately disturbed (forested habitats fragmented by roads and trails) **(2)**; the subpopulations are located in areas subject to periodical vegetation clearing (trails, road and including uses of herbicides along PR-120) **(1)**; the subpopulations are located in areas subject to stochastic events (e.g., fire, severe drought, hurricanes, among others) **(1)**; and population size is relatively high (more than 20 imagoes and more than 500 larvae per ha) **(12)**. Therefore, the

PRHB population in the Maricao Commonwealth Forest is considered to have **moderately high resiliency (19)**.

5.2.5 Current Resiliency of the Susúa Commonwealth Forest Population

The Susúa Commonwealth Forest is a public land administered by PRDNER for its conservation. The Forest is recognized as one of the Puerto Rico's Critical Wildlife Areas (PRDNER 2005, p. 275). The habitats on which the PRHB depends in the Susúa Commonwealth Forest are protected under the *Ley de Bosques de Puerto Rico* (Puerto Rico Forests' Law), as amended in 2000.

In the Susúa Commonwealth Forest habitat protection is substantial **(3)**; the subpopulations are located within 1 km of each other, and the habitat between subpopulations is moderately disturbed (forested habitats fragmented by roads and trails) **(2)**; the subpopulations are located in areas subject to periodic vegetation clearing (trails and road) **(1)**; the subpopulations are located in areas subject to stochastic events (e.g., fire, severe drought, hurricanes, among others) **(1)**; and population size is relatively low with a high degree of uncertainty in population trends **(4)**. Therefore, the PRHB population in Susúa Commonwealth Forest is considered to have **low resiliency (11)**.

5.3 Current Resiliency Summary

There are five extant PRHB populations.. We classified current resiliency as moderately high in one (1) population, moderate in three (3) populations, and low in (1) population (Table 5-2). Our classifications of resiliency rely on habitat characteristics and population size or trend estimates. The population with moderately high resiliency (Maricao Commonwealth Forest) occurs in land managed for conservation, but in this forest the species occurs at edges of trails and roads where vegetation is frequently removed and herbicide applied. Therefore, anthropogenic activities may negatively affect the status of the species. The population in IQC has moderate resiliency, although it occurs in areas where anthropogenic activities may negatively affect the species, because it has the largest known PRHB population size. The populations in Río Abajo Commonwealth Forest and the Río Encantado Area have moderate resiliency because they occur in habitats managed for conservation that are protected from development and other anthropogenic activities. The Susúa Commonwealth Forest population has low resiliency Although this forest is managed for conservation, the species occurs at edges of or along trails where vegetation is frequently removed and herbicide applied, and the PRHB population size is low. Overall (rangewide), PRHB population resiliency is currently moderate, based on our index (11 + 19 + 18 + 15 + 14 = 77 ÷ 5 = 15.4).

Table 5-2. Summary table of the five assessed Puerto Rican harlequin butterfly populations and factors that contribute to their resiliency classification. Values reflecting good conditions for the butterfly are shaded green, while values reflecting unfavorable conditions are shaded orange, and moderate values are shaded yellow.

Population	Habitat Metrics				Population Metric	Resiliency
	Habitat Protection	Connectivity/ Habitat Fragmentation	Vegetation Clearing/Use of Pesticides	Susceptibility to Stochastic Events	Population Size/Trend Description	
Isabela Quebradillas and Camuy (IQC)	<34 percent protected **(1)**	Both **(2)**	High **(1)**	Moderate **(2)**	More than 25 imagoes and more than 500 larva per ha, and/or growth **(12)**	Moderate **(18)**
Rio Abajo Commonwealth Forest	Most habitat protected (>66 percent) **(3)**	Connectivity **(3)**	Low **(3)**	Moderate **(2)**	Relatively low population size (0 to 5 imagoes and less than 100 larvae per ha) or high degree of uncertainty in population size/trends **(4)**	Moderate **(15)**
Rio Encantado	Some habitat protected (34-66 percent) **(2)**	Connectivity **(3)**	Low **(3)**	Moderate **(2)**	Relatively low population size (0 to 5 imagoes and less than 100 larvae per ha) or high degree of uncertainty in population size/trends **(4)**	Moderate **(14)**
Maricao Commonwealth Forest	Protected **(3)**	Both **(2)**	High **(1)**	High **(1)**	Relatively high population size (>20 imagoes and > 100 larvae per ha) and/or growth **(12)**	Moderately High **(19)**
Susúa Commonwealth Forest	Protected **(3)**	Both **(2)**	High **(1)**	High **(1)**	Low population size with high degree of uncertainty in population trends **(4)**	Low **(11)**

5.4 Current Redundancy

Redundancy is reflected by the distribution of populations across life zones and the degree to which the spatial arrangement of populations enables the species to persist after stochastic events, such as hurricanes, severe drought or wildfires (Table 5-3). Because the PRHB is a narrow ranging endemic, redundancy is inherently low The exact historic distribution of the PRHB is unknown, but it is presently disjointed, suggesting populations were once more widely distributed across the Island. This reduction in distribution, could be attributed to wide scale habitat destruction and other factors that have isolated and extirpated populations. Currently, with five (5) very small populations and only one (1) of those considered to have moderately high resilience, the species is not well buffered against the effects of stochastic events. Moreover, conversion of forested habitats for agriculture and urbanization could continue affecting existing populations and the habitat in the current range of the species. Additional habitat conversion, incompatible management practices, and other stressors have further eroded the species redundancy by reducing the number of populations and the geographic area inhabited by the species.

Table 5-3. Current Distribution, Number of Populations and Subpopulations, and Population Index for the Puerto Rican harlequin butterfly.

Region of Puerto Rico	Population	Number of Meta-populations	Population Index	Surveyed Area	Source of Information
Northern Karst Region	Isabela, Quebradillas and Camuy	13	Less than 50 imagoes/10 to 100 larva	1.3 ha (3.2 ac)	(Carrión-Cabrera 2003, p. 34) (Monzón-Carmona 2007, p. 44) (Biaggi-Caballero 2010, p. 4)
			Less than 60 imagoes/ approximately 1,400 larva	2.68 ha (6.67 ac)	Barber 2018, p. 1)
	Río Encantado Area	3	Not determined (imagoes, larvae and chrysalids)	Unknown	Morales and Estremera 2018, p. 1
	Río Abajo Commonwealth Forest	1	Not determined (imagoes, larva and chrysalis)	Unknown	Morales and Estremera 2018, p. 1

Table 5-3 continued

Region of Puerto Rico	Population	Number of Meta-populations	Population Index	Surveyed Area	Source of Information
Central-western Volcanic-Serpentine Region	Maricao Commonwealth Forest	3	12 imagoes/ no data about larvae	Not determined (unknown)	Asencio 1984, p. 1
			No more than 5 imagoes / no more than 10 larvae	Not determined (unknown)	(Carrión-Cabrera 2003, p. 48), (Pérez-Asso et al. 2009, p. 94)
			21 imagoes/631 larvae	1.08 ha (2.67 acres)	(Barber 2018, p. 1)
	Susúa Commonwealth Forest	2	Unknown (Not counted since 1980's)	Not determined (unknown)	(Biaggi-Caballero 2010, p. 4).
			16 adults (imago)/83 larvae	1.08 ha (2.67 acres)	(Barber 2018, p. 1)
Southern Karst Region	Tallaboa Guayanilla/Peñuelas	1	Unknown (Not observed since 1926)	Unknown	(Biaggi-Caballero 2010, p. 4)

5.5 Current Representation

The PRHB representation is influenced by the breadth of adaptive diversity possessed by the species and by maintaining the evolutionary processes (i.e., gene flow and natural selection) that drive adaptation. Representation improves with increased genetic and/or ecological diversity within and among populations. Presently there is substantial uncertainty regarding representation for this species due to lack of knowledge on genetic diversity, adaptive potential and differences between the PRHB populations. Currently, representation appears to be moderate to high because the PRHB occurs in four ecological settings or life zones. Thus, the PRHB seems to have the capability to adapt to different landscapes as long as the fundamental needs for nesting (host plant) and foraging are met.

5.6 Summary of PRHB Condition based on the 3Rs

There is sufficient information to conceptualize and estimate the condition of the 3Rs for the PRHB (Figure 5-1). Currently PRHB populations rangewide have representation in two (2) geographic regions and four (4) life zones. There are five (5) metapopulations that serve as a measure of species redundancy. One (1) of those metapopulations has moderately high resiliency, three (3) have moderate resiliency, and one (1) has low resiliency.

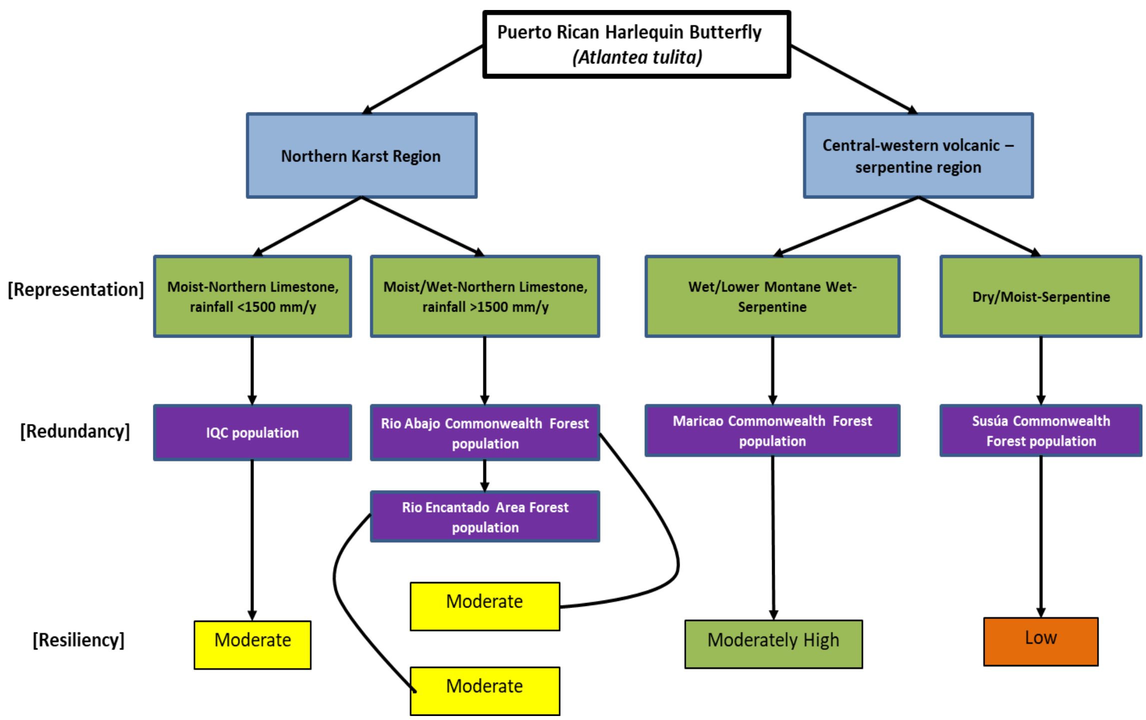


Figure 5-1. Basic conceptual model showing how the Puerto Rican harlequin butterfly is represented (life zones), its redundancy (populations) and its resiliency.

CHAPTER 6- FUTURE CONDITION SCENARIOS

6.1 Introduction

In this chapter, we describe our analysis of the future conditions for the PRHB. To capture and categorize the range of realistic future conditions, we considered the following generalized scenarios for each population: a) best case; b) worst case; and c) most likely case. These scenarios match the most recent climate change scenarios described for Puerto Rico (Henareh et al. 2016, entire). Our analyses relied on available data, expert judgments regarding the consequences of interacting influences, and our assessment of likely future habitat conditions. Because we do not fully understand the interacting causal relationships and are unable to predict future habitat conditions with certainty, our analyses are necessarily predicated upon numerous assumptions. We identify these fundamental assumptions used and discuss the implications of these assumptions in this Chapter.

To analyze species' viability, we considered the current and future availability or condition of resources that the PRHB rely on (see Chapter 3, Section 3.4, Summary of Ecological Needs). The range of what may happen in each scenario is described based on the current condition and how resiliency, representation, and redundancy would be expected to change. For this assessment, we defined viability as the ability of a species to sustain itself over time. To maintain viability, a species must have sufficient abundance and distribution to withstand changes in its biological and physical environment, and environmental stochasticity (e.g., heavy rains, drought).

We chose 25 years as the time frame for the PRHB analysis because this timeframe includes at least 25 generations, thus allowing adequate time to detect population and habitat trends. Our predictions associated with this time frame also are supported by existing predictive models regarding regional climate change. In particular, potential impacts associated with changing climatic conditions (e.g., estimates for precipitation and drought levels) were based on published climate model projections downscaled for Puerto Rico and the Virgin Islands (Henareh et al. 2016, entire).

6.2 Future Habitat Loss and Fragmentation by Urban Development

The primary stressor affecting the future condition of the PRHB is habitat loss associated with urban development (Figure 6-1; Table 6-1) and other land use changes (e.g., agriculture and cattle rearing). These stressors account for direct and indirect effects at some level to all life stages and across the species' range. Additive habitat loss stressors projected for the future also include habitat modification by roadside vegetation clearing, use of pesticides and climate change. Additionally, we consider the susceptibility of the species habitat to catastrophic events (i.e., human-induced fires). All these stressors are predicted to result in alterations of habitat suitability for the species, which may adversely affect the resiliency, redundancy and representation of the PRHB.

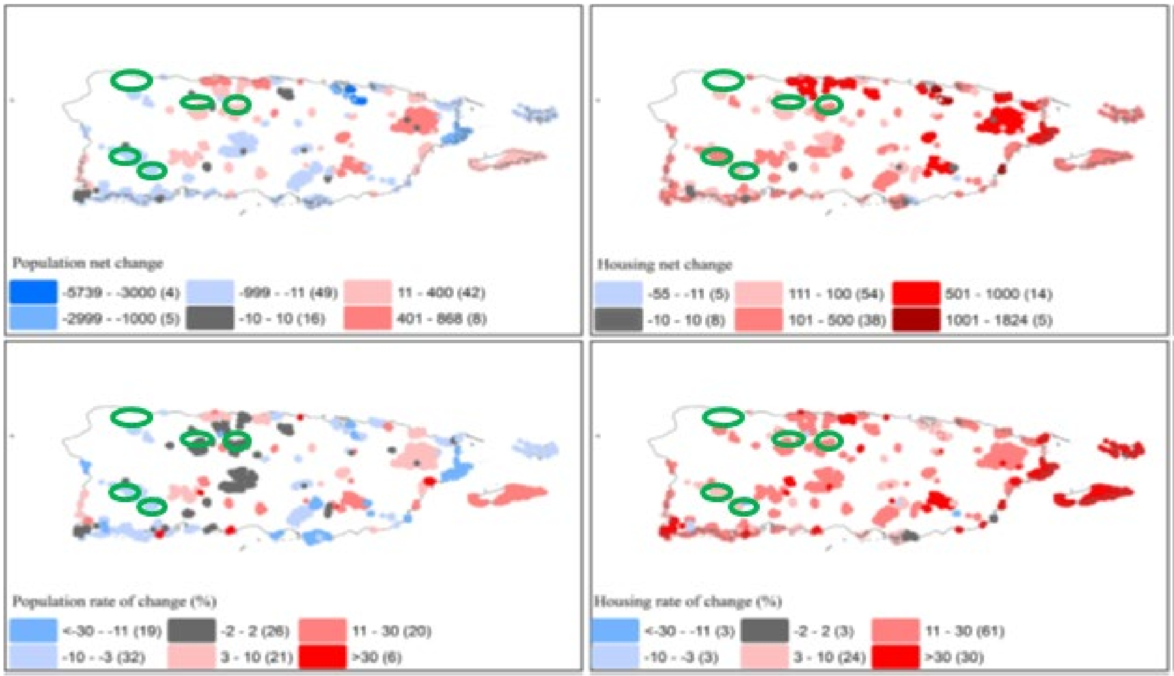


Figure 6-1. Spatial patterns of housing and human population changes within 1 km of protected areas. The number of protected areas in each class is shown in parentheses (Castro-Prieto 2017, p. 477). Green circles indicate the location of the PRHB populations in Puerto Rico.

Table 6-1. Projected housing net change within a 1 kilometer buffer zone around Puerto Rican harlequin butterfly populations from 2020 to 2045. Data derived from Castro-Prieto et al. (2017, pp. 478-479). Worst Case assumes a continuation of urban growth observed during 2000-2010; Most Likely assumes half of observed past growth; Best Case assumes no future growth.

Population	Scenario		
	Worst Case	**Most Likely**	**Best Case**
IQC	8 percent per decade	4 percent per decade	0 percent per decade
Río Abajo	16 percent per decade	8 percent per decade	0 percent per decade
Río Encantado	16 percent per decade	8 percent per decade	0 percent per decade
Maricao	9 percent per decade	4.5 percent per decade	0 percent per decade
Susúa	6 percent per decade	3 percent per decade	0 percent per decade

6.3 Future Climate Conditions

Temperature, drought, and storm frequency and intensity are projected to increase based on climate models. Concomitant changes in the spatial distribution of life zones in Puerto Rico also are expected as a result of climate change.

6.3.1 Temperature

Temperatures in the U.S. Caribbean have fluctuated over the last 100 years; however, since 1950 temperatures have increased by about 1.5°F (0.83°C) in Puerto Rico (Figure 6-2; USGCRP 2018, 20:819). In this section we present temperature in Fahrenheit degrees first, as published in the USGS source we used. Some climate projections (1960-2099) indicate a 4.6 to 9°C (8.3 to 16.2°F) temperature increase for Puerto Rico (Figure 6-3; Henareh et al. 2016, p. 275) indicating a general consensus on a continued warming trend into the future amongst climate modeling studies for the entire U.S. Caribbean including the USVI. Thus, temperature across the Caribbean is expected to continue increasing over the next century. Global climate models project about a 1.5°F (0.83°C) to 4°F (2.2°C) increase in average temperatures for the U.S. Caribbean in 30 years (year 2050) with the end of the century (2100) estimates showing increases as high as about 9°F (4.9°C) under higher emission scenarios (USGCRP 2018, 20:819). Major consequences of warming, also include significant increases in the number of days in the Caribbean with temperatures over 90°F (32.2°C). For example, since 1970, the average annual number of days exceeding 90°F (32.2°C) has gone up an average of 0.5 days per year (USGCRP 2018, 20:821).

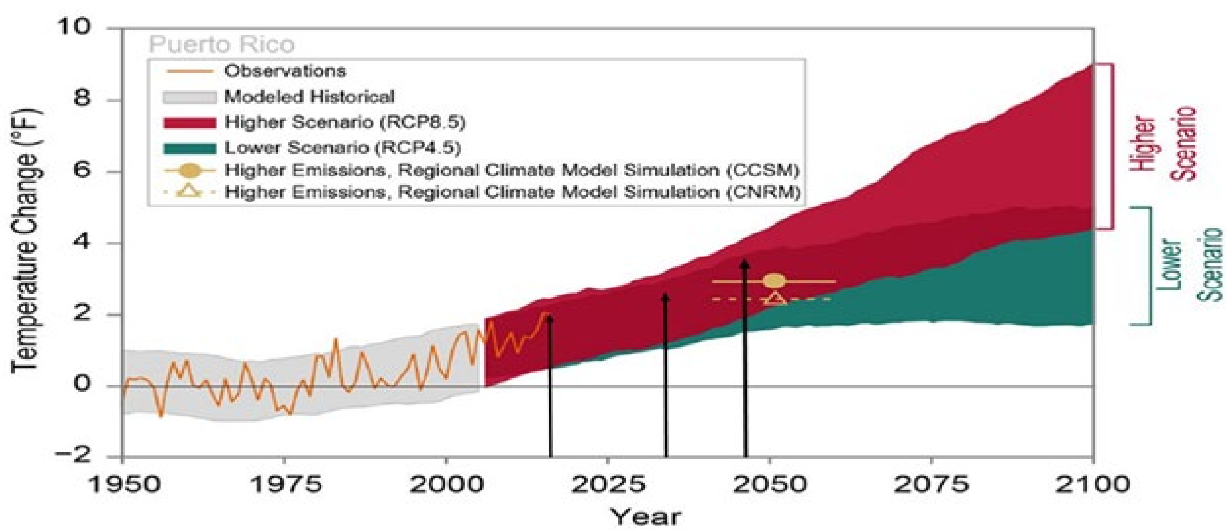


Figure 6-2. Observed and projected temperature changes are shown compared to the 1951-1980 average. Observed data are for 1950-2017, and the range of model simulations for the historical period is for 1950-2005. The range of projected temperature changes from global climate models is shown for 2006-2100 under lower and higher emissions scenarios. Projections from two regional climate models are shown for 2036-2065, and they align with those from global models

for the same period (USGCRP 2018, 20:820). Black arrows denote temperature at current time, an increase at 10 (2030) and 25 (2045) years into the future. Y-axis (data) indicates temperature; X-axis (index) indicates 25-year increments from 1950.

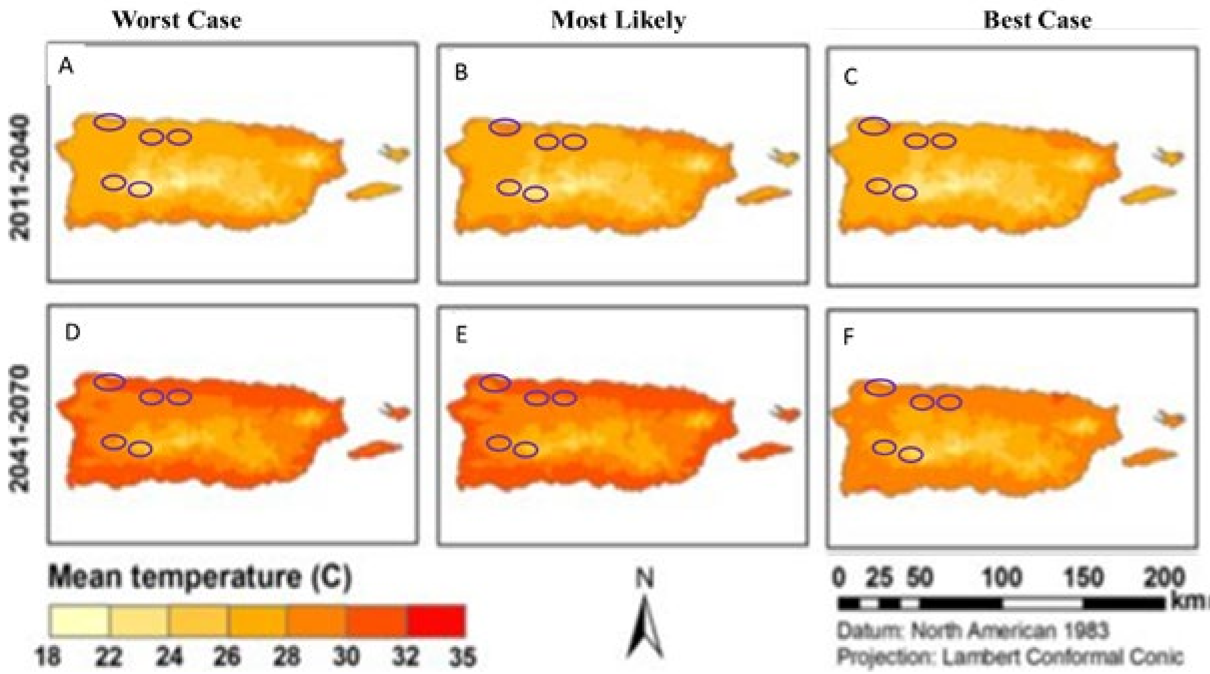


Figure 6-3. Temperature projections for Puerto Rico under three general circulation models (GCM) scenarios (Henareh et al. 2016, p. 277). Purple circles indicate the locations of the Puerto Rican harlequin butterfly populations. Figures A, B and C show current condition and the projection for 20 years (2040); Figures D, E and F show the projection at 50 years (2070).

6.3.2 Precipitation and drought stress.

Precipitation is projected to decrease relative to current levels, which combined with further warming, will tend to accelerate the hydrological cycle, resulting in more frequent wet and dry extremes (Jennings et al. 2014, p. 4; Cashman et al. 2010, p. 1). Indeed, the majority of models predict that future decreases in precipitation are likely (Carter et al. 2014, p. 399). Thus, the Caribbean is expected to get more frequent and severe droughts from reduced precipitation and increased evapotranspiration ratio (Figure 6-4) with a concomitant increase in the amount of precipitation produced during hurricane events (Herrera et al. 2018, p. 1). Subtropical dry forests inherently tend to be subject to water deficit for ten months of the year (Miller and Lugo 2009, p. 86) and are expected to become even drier in the future, especially in regions like the U.S. Caribbean (USGCRP 2018, 20: 820). Climate models consistently project significant drying in the U.S. Caribbean region occurring by the middle of the century; that is, by our projected time horizon of 2045 (USGCRP 2018, 20: 820). Although heavy rainfall associated with hurricanes is expected to increase, shifting weather patterns have nevertheless caused total rainfall to decrease in the Caribbean, resulting in more pronounced seasonal droughts (EPA report, 2016, p. 1).

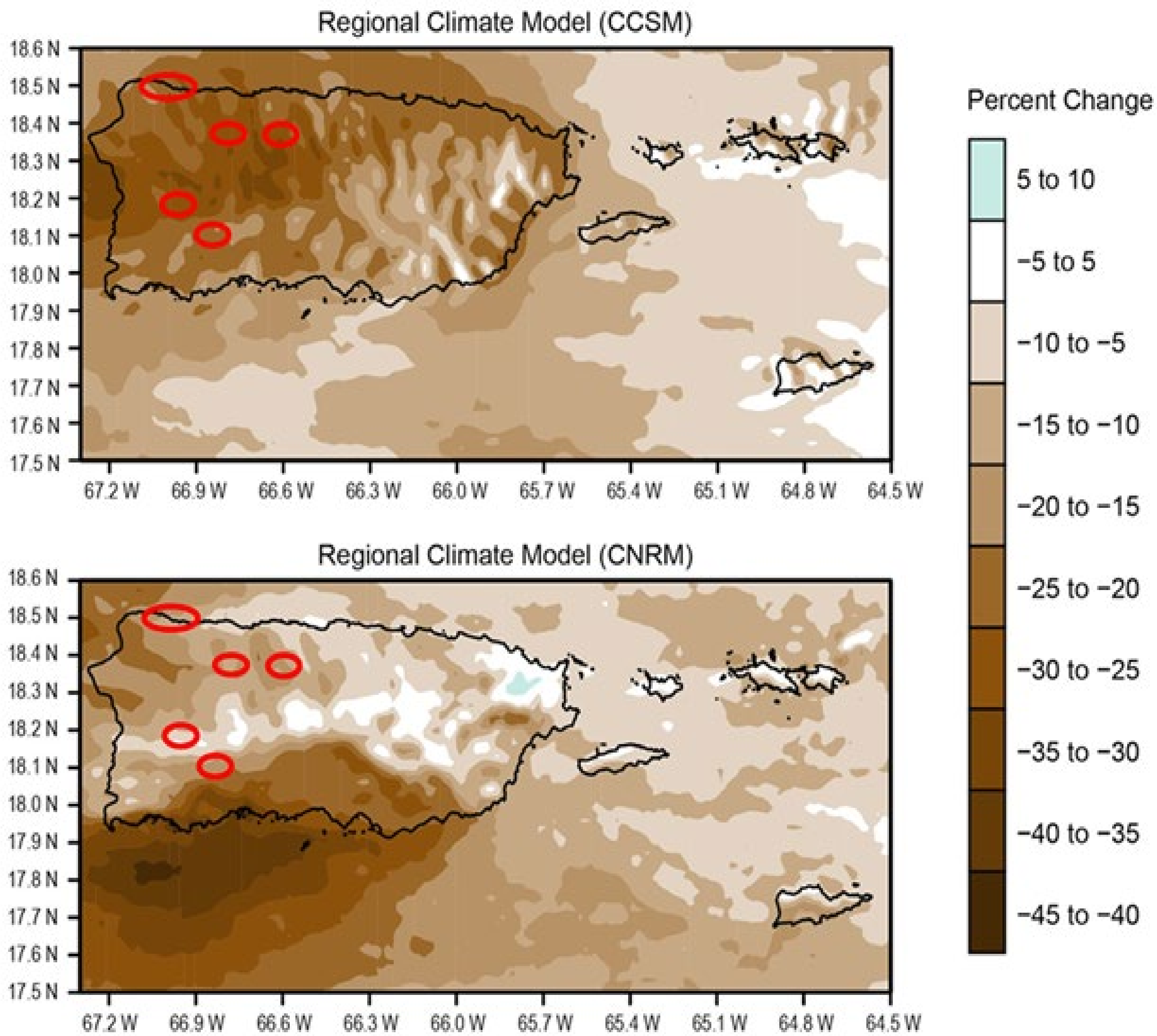


Figure 6-4. Projected Precipitation Change for the U.S. Caribbean. This figure shows the projected percent change in annual precipitation over the U.S. Caribbean region for the period 2040-2060 (lower figure) compared to 1985-2005 (upper figure) based on the results of two regional climate model simulations. These simulations downscale two global models for the higher scenario and show that within-island changes are projected to exceed a 10 percent reduction in annual rainfall. Red circles indicate the locations of the PRHB populations in Puerto Rico.

While we currently do not know the maximum thermal tolerance of the PRHB, studies with other species of Lepidopterans have shown that increases in ambient temperature are associated both with changes in metabolic rates and declines in reproductive success (e.g., Mathavan and Pandian 1975, entire; Koda and Nakamura 2010, entire; Wong et al. 2016, entire). For example, Koda and Nakamura (2010, p. 30) reported that hatchability of *Shijimiaeoides divinus barine* butterfly eggs steadily decreased from 88 percent to 0 percent with increases in ambient temperatures from 20º to 35ºC (68 to 95ºF). Under past and current environmental conditions, reproduction of the PRHB occurs in environments with annual average maximum temperatures of 82-90ºF (28-32ºC); Table 11. However, based on our future climate projections (Table 11), these temperatures are expected to increase by 2.8-3.3ºC (5.04-5.94ºF) ("Best Case Scenario") to 4.6-5.5ºC (8.28-9.9ºF) ("Worst Case Scenario"), resulting in maximum temperatures ranging from approximately 89-98ºF (31-36ºC) ("Most Likely Scenario") for all known PRHB populations by 2045. Although the effects of this temperature increase on reproductive success of the PRHB remain uncertain, the cited studies suggest that PRHB reproduction may be adversely affected. Moreover, given that egg-laying and subsequent larval growth of the PRHB is closely associated with new leaf growth of *O. spinosa* triggered by the onset of the rainy season (ca. May-June; Figures 13 and 14), any future climatic aberrations which disrupt or reduce such rains will also likely have a detrimental effect on PRHB reproduction. It is conceivable that an extended drought (as predicted by the MCDD projections, Figure 6-5; Table 6-2) during the rainy season could prevent the species from reproducing in the areas affected, potentially resulting in localized population extirpations.

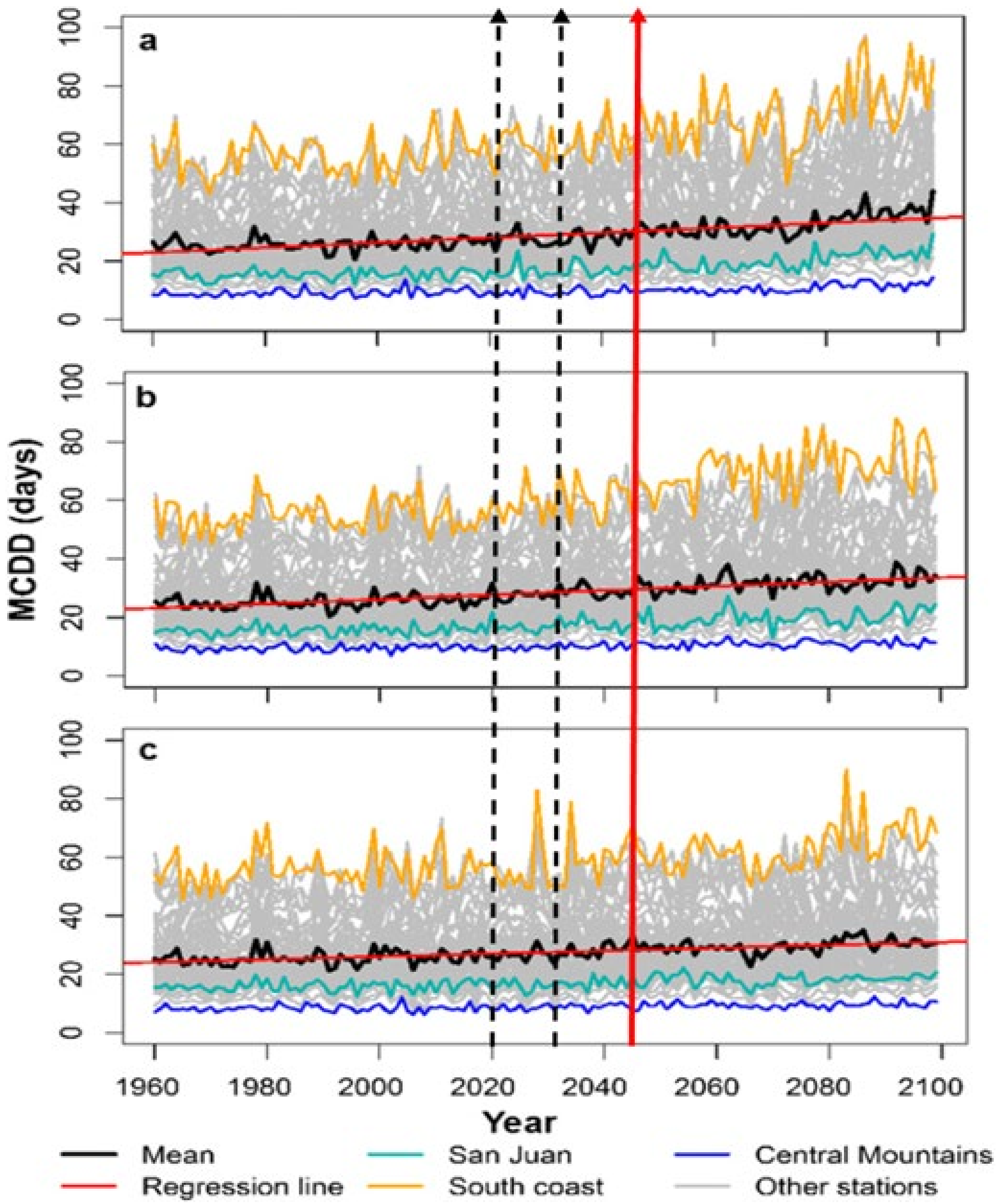


Figure 6-5. Maximum number of consecutive dry days (MCDD) in Puerto Rico. Black arrows represent present time (2020) and 10 years into the future. Red arrow indicates MCDD at 25 years into the future (2045). Panel A represents "worst case scenario"; B represents "most likely scenario"; C represents "best case scenario." Y-axis (data) indicates days; X-axis (index) indicates 20-year increments from 1960 (adapted from Henareh et al. 2016, p. 276).

Table 6-2. Predicted increases in temperature and maximum consecutive dry days in Puerto Rico by 2045, based on Henareh et al. (2016). Because projections of Henareh et al. (2016) were for 1960-2099, we used 61 percent of their overall projection (85/139 yrs) and assumed a linear trend to estimate temperature increases for 2045. Maximum consecutive dry days were based on interpolation of mean plots in Henareh et al. (2016; Figure 7, p. 276).

Projection to 2045	**Best Case**	**Worst Case**	**Most Likely**
Temperature increase	2.8-3.3℃	4.6-5.5℃	3.9-4.6℃
Maximum Consecutive Dry Days	24 days	32 days	27 days

6.3.3 Life Zones

The boundaries of life zones according to the Holdridge System are based on three climatic measurements: annual precipitation, biotemperature and ratio of potential evapotranspiration to annual precipitation (Holdridge 1947, entire; Ewel and Whitmore 1973, p. 4). Figure 3-3 (Chapter 3) illustrates the distribution of the major life zones in Puerto Rico. Dramatic shifts in several life zones in Puerto Rico with potential loss of subtropical rain, moist, and wet forest, and the appearance of tropical dry and very dry forests are anticipated during this century (Henareh et al. 2016, p. 275). In the case of restricted range species, such as the PRHB, these trends may lead to biome shifts and species range loss due to inability of such species to effectively migrate or adapt to these changes (IPCC Report 2018, p. 3: 128). In fact, the number of plant and vertebrate species projected to lose over half of their climatically determined geographic range effectively doubles at 2°C versus 1.5°C of warming (IPCC report, 2018, p. 3:8). However, for insect species this number is effectively tripled by a 2°C temperature increase.

Life zone distribution changes are predicted in Puerto Rico (Figure 6-6; Henareh et al. 2016, p. 277) resulting from the predicted future temperature, precipitation and drought stress conditions. Overall, the current life zones where the PRHB occurs will most likely experience higher temperatures, reduced precipitation and increased drought stress conditions, thus reducing their suitability to sustain the species, thereby potentially resulting in localized extirpations of the species.

Consequently, the capacity of the PRHB to adapt to such conditions is expected to be reduced due to the current small number of populations and individuals.

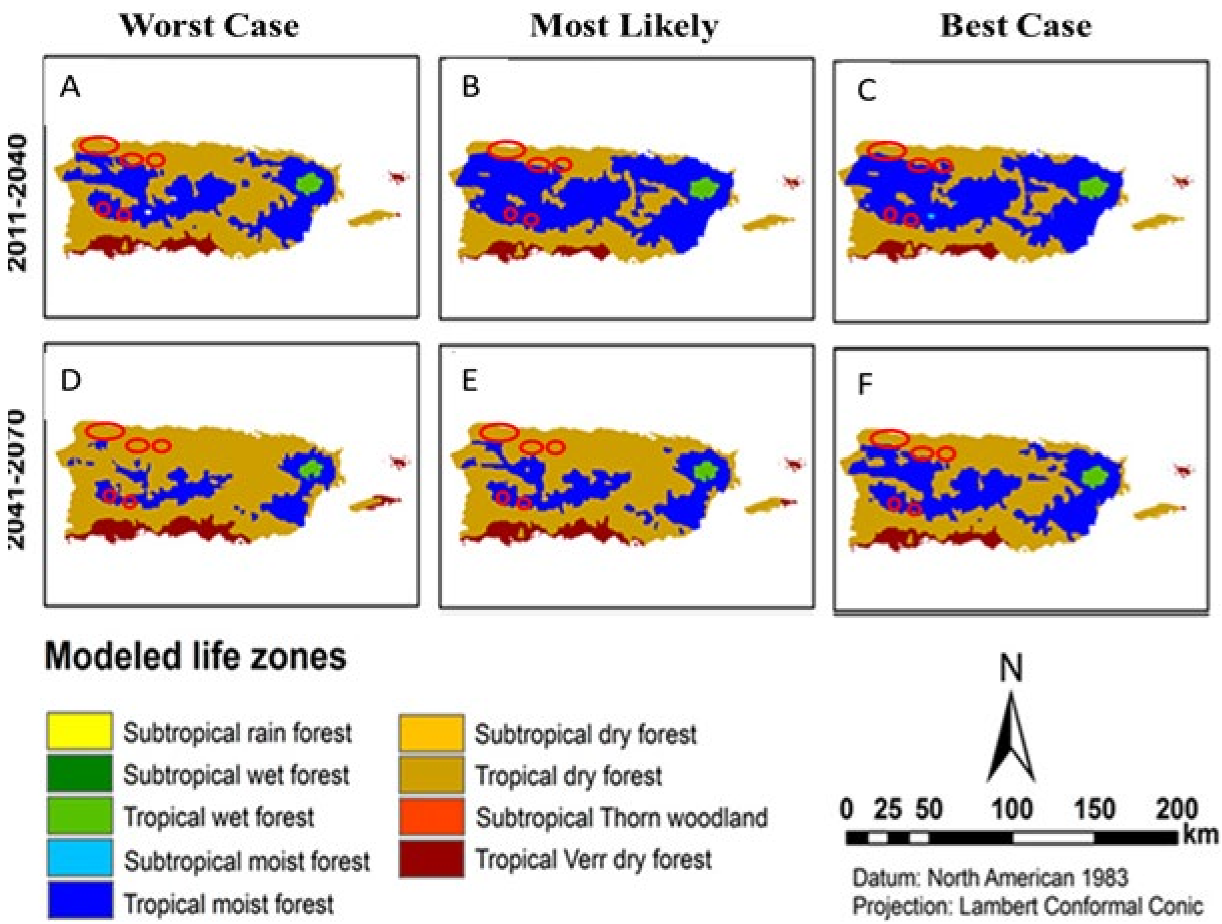


Figure 6-6. Projected life zones from the average of all models under the three future scenarios. Red circles indicate currently known populations of PRHB. Figures A, B and C show current condition; Figures D, E and F show the projection at 25 years (2045) and beyond. Adapted from Henareh et al. (2016), p. 279.

6.3.4 Storm Frequency and Intensity

Reconstruction of the past 5,000 years of intense hurricane activity in the western North Atlantic suggests that hurricane variability has been strongly modulated by El Niño during this time, and that the past 250 years has been relatively active in the context of the past 5,000 years (PRCCC Report 2013, p. 31). Accordingly, hurricanes may play an important role in shaping forest structure within the Caribbean (Van Bloem et al., 2005 p. 571; Lugo 2008, p. 368; Feng et al. 2018, p. 2). However, extreme events such as major hurricanes, floods and droughts are projected to increase in frequency and intensity, particularly in the Caribbean region (USGCRP 2018, 20: 127). Indeed, tropical storms and hurricanes have become more intense during the past 20 years, and hurricane wind speeds and rainfall are likely to increase further as the climate continues to warm. According to regional climate projections by Bender et al. (2010, entire), the frequency of intense (Categories 4-5) hurricanes is expected to increase approximately 1 percent per year over this century. Increasing hurricane intensity and frequency coupled with a species showing reduced populations, low number of individuals, habitat degradation and fragmentation would likely have adverse consequences both for the PRHB and its habitat.

Long-term viability will require resilient populations in locations that are protected from long-term catastrophic but permanent effects of climate change (e.g., catastrophic hurricanes claiming forested habitat). The lack of redundancy in the face of hurricane threats is well illustrated by the path of Hurricane Maria in 2017 (Figure 6-7) and other historical hurricanes (Figure 6-8). Hurricane Maria traversed Puerto Rico in northwest direction, exiting near the city of Arecibo, and causing widespread destruction across the island. The entire range of the PRHB was subjected to hurricane force winds (> 64 knots) as the hurricane passed over, first as a Category 5 hurricane, weakening to a Category 4 hurricane over the Puerto Rico mainland. Feng et al. (2018, p. 2) estimated that Hurricane Maria may have caused mortality and severe damage to 23-31 million trees in Puerto Rico.

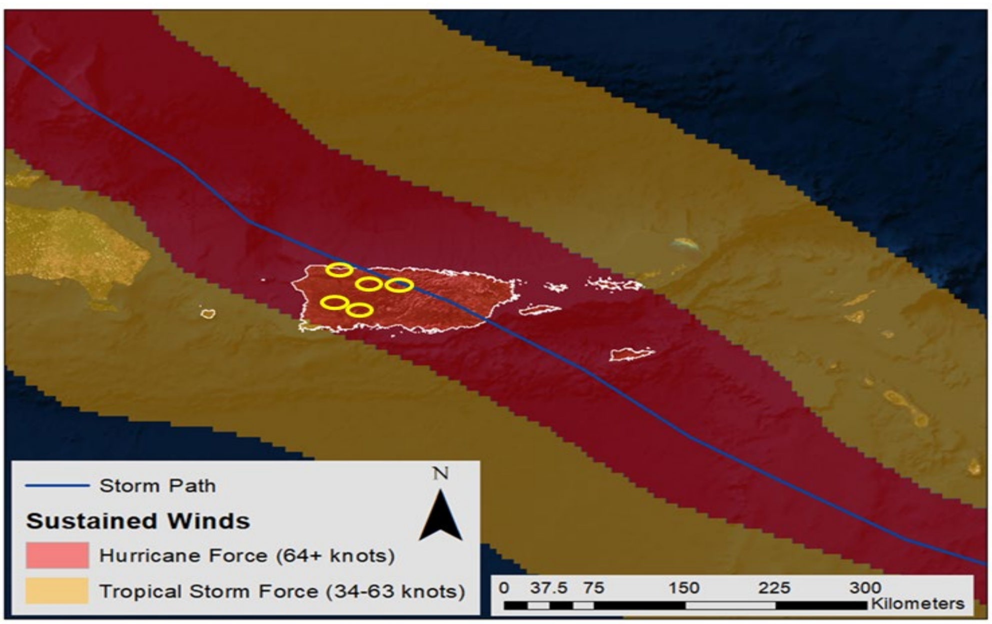


Figure 6-7. Path and wind speed of Hurricane Maria in September 2017. Puerto Rico and the US Virgin Islands are outlined in white, and the approximate range of the Puerto Rican harlequin butterfly is circled in yellow. (Data accessed from National Hurricane Center, National Oceanic and Atmospheric Administration, https://www.nhc.noaa.gov, March 27, 2018)

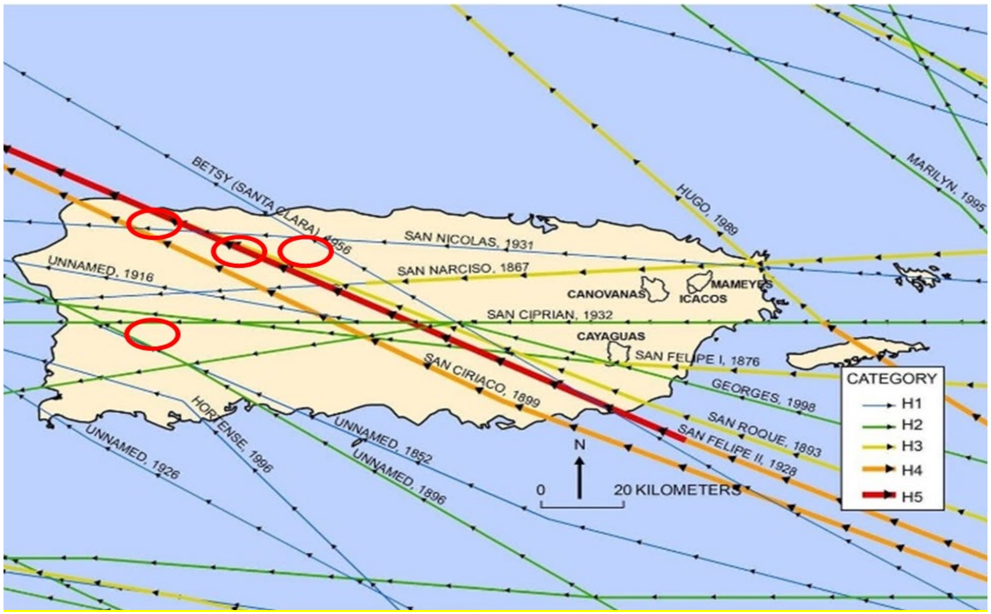


Figure 6-8. A sample of historical hurricanes that have struck Puerto Rico (USGS, public domain https://www.usgs.gov/media/images/puerto-rico-hurricanes-map), red circles identify the current locations of the Puerto Rican harlequin butterfly populations (Adapted from Feng et al. 2018, p. 3).

6.4 Future Scenarios

Resiliency was scored (Table 6-3) by combining scores for four (4) habitat metrics (Protection/Development Risk, Connectivity/Habitat Fragmentation, Risk of Vegetation clearing/Use of Pesticides, and Susceptibility to Stochastic Events (e.g., human-induced fires, severe drought, hurricanes, among others) and one (1) population metric (population size or trend). For future conditions scenarios, expected changes in habitat are based on scientific data and published documents. The projected population metric is less influential because here it reflects condition of the habitat and, unlike in current condition, it is not a direct measure. The habitat metrics are the drivers that may promote changes in future population (unless the current population is so low that extirpation risk is high). Therefore, habitat metrics had more weight than the population metric in future conditions projections.

For future conditions, each habitat metric was assigned a score of one (1), two (2), or three (3), and each population metric was assigned a score of two (2), four (4), or six (6), as described below in Table 6-3. Habitat metrics were weighted equally, with the overall effect that "habitat" was weighted two (2) times higher than "population."

Table 6-3. Habitat and population metric values to project future resiliency of Puerto Rican harlequin butterfly populations.

H Score	Habitat Metrics Influencing Viability				Population Metrics	P Score
	Habitat Protection	Connectivity/Habitat Fragmentation	Vegetation Clearing/Use of Pesticides	Susceptibility to Stochastic Events	Population size/Trends	
1 point each; 4 points totals	Most habitat not protected, at risk of being developed (<34 percent protected)	Isolated subpopulations located at a distance of more than 1 km from the next one; habitat between populations or subpopulations highly disturbed (low connectivity)	Subpopulations located in areas subjected to vegetation clearing (including the use of herbicides) and use of pesticides (mosquito control and agricultural practice)	Subpopulations located in areas more vulnerable to stochastic events (e.g., fire, severe drought, hurricanes, among others)	Relatively low population size (0 to 5 imago and less than 100 larvae per ha) or high degree of uncertainty in population size/trends	2
2 points each; 8 points total	Some habitat protected, with some at risk of being developed (34-66 percent protected)	Subpopulations located within 1 km range; habitat between population moderately disturbed (some forested corridors)	Subpopulations located in areas rarely occur vegetation clearing (including the use of herbicides) or use of pesticides (mosquito control and agricultural practice)	Subpopulations located in areas with moderate vulnerability to stochastic events (e.g., fire, severe drought, hurricanes, among others)	Relatively moderate population size (6 to 20 imagoes and 100 to 500 larvae per ha)	4
3 points each; 12 points total	Most habitat protected (>66 percent)	Subpopulations located within 1 km range; un-disturbed habitat between populations (forested corridors)	Subpopulations located in areas where vegetation clearing (include the use of herbicides) or use of pesticides (mosquito control and agricultural practices) is not expected.	Subpopulations located in areas with lower vulnerability to stochastic events (e.g., fire, severe drought, hurricanes, among others).	Relatively high population size (more than 20 imagoes and more than 500 larvae per ha) and/or growth.	6

The score for each population across all metrics were summed, and final population resiliency categories were assigned as follows:

Low Resiliency:	≤ 9
Moderately Low Resiliency	9 to 10
Moderate Resiliency:	11 to 13
Moderate High Resiliency:	14 to 15
High Resiliency:	≥ 15

Projected population resiliency under each of the three scenarios is shown in Tables 6-4, 6-5, and 6-6.

Table 6-4. Worst Case Scenario for future conditions (2045).

Population	Habitat Metrics				Population Metric	Resiliency
	Habitat Protection - Development Risk	Connectivity Habitat Fragmentation	Risk of Vegetation Clearing/Use of Pesticides (Low, Moderate or High)	Susceptibility to Stochastic Events (Low, Moderate or High)	Population Size/Trend Description	
Isabela, Quebradillas and Camuy (IQC)	Risk of Development (1)	Habitat fragmented / low connectivity (1)	High (1)	High (1)	Relatively low population size (0 to 5 imago and at less than 100 larvae per ha) or high degree of uncertainty in population size/trends (2)	Low (6)
Rio Abajo Commonwealth Forest	Protected (3)	Connectivity (3)	Low (3)	High (1)	Extirpated	Extirpated
Rio Encantado Area	Some habitat protected, some at risk of being developed (1)	Both (2)	Moderate (2)	High (1)	Extirpated	Extirpated

Table 6-4 continued

Population	Habitat Metrics				Population Metric	Resiliency
	Habitat Protection - Development Risk	Connectivity Habitat Fragmentation	Risk of Vegetation Clearing/Use of Pesticides (Low, Moderate or High)	Susceptibility to Stochastic Events (Low, Moderate or High)	Population Size/Trend Description	
Maricao Commonwealth Forest	Protected **(3)**	Habitat fragmented / low connectivity **(1)**	High **(1)**	High **(1)**	Relatively low population size (0 to 5 imago and less than 100 larvae per ha) or high degree of uncertainty in population size/trends **(2)**	Moderately Low **(8)**
Susúa Commonwealth Forest	Protected **(3)**	Habitat fragmented / low connectivity **(1)**	High **(1)**	High **(1)**	**Extirpated**	**Extirpated**

Table 6-5. Most Likely Scenario for future conditions (2045).

Population	Habitat Metrics				Population Metric	Resiliency
	Habitat Protection - Development Risk	Connectivity Habitat Fragmentation	Risk of Vegetation Clearing/Use of Pesticides (Low, Moderate or High)	Susceptibility to Stochastic Events (Low, Moderate or High)	Population Size/Trend Description	
Isabela, Quebradillas and Camuy (IQC)	Risk of Development **(1)**	Habitat fragmented / low connectivity **(1)**	High **(1)**	High **(1)**	Relatively moderate population size (15 to 20 imagoes and 100 to 500 larvae per ha) **(4)**	**Low (8)**
Rio Abajo Commonwealth Forest	Protected **(3)**	Connectivity **(3)**	Low **(3)**	High **(1)**	**Extirpated**	**Extirpated**
Rio Encantado Area	Some habitat protected, some at risk of being developed **(2)**	Connectivity **(3)**	Low **(3)**	Moderate **(2)**	**Extirpated**	**Extirpated**

Table 6-5 continued

Population	Habitat Metrics				Population Metric	Resiliency
	Habitat Protection - Development Risk	**Connectivity Habitat Fragmentation**	**Risk of Vegetation Clearing/Use of Pesticides** (Low, Moderate or High)	**Susceptibility to Stochastic Events** (Low, Moderate or High)	**Population Size/Trend** Description	
Maricao Commonwealth Forest	Protected **(3)**	Both **(2)**	High **(1)**	High (1)	Relatively moderate population size (15 to 20 imagoes and 100 to 500 larvae per ha) **(4)**	**Moderately Low (10)**
Susúa Commonwealth Forest	Protected **(3)**	Both **(2)**	High **(1)**	High **(1)**	**Extirpated**	**Extirpated**

Table 6-6. Best Case Scenario for future conditions (2045).

Population	Habitat Metrics				Population Metric	Resiliency
	Habitat Protection - Development Risk	**Connectivity Habitat Fragmentation**	**Risk of Vegetation Clearing/Use of Pesticides** (Low, Moderate or High)	**Susceptibility to Stochastic Events** (Low, Moderate or High)	**Population Size/Trend** Description	
Isabela, Quebradillas and Camuy (IQC)	Risk of Development **(1)**	Both **(2)**	High **(1)**	Moderate **(2)**	Relatively high population size (more than 25 imagoes and more than 500 larvae per ha) and/or growth **(6)**	**Moderate (12)**
Rio Abajo Commonwealth Forest	Protected **(3)**	Connectivity **(3)**	Low **(3)**	Moderate **(2)**	Relatively low population size (0 to 5 imago and at less than 100 larvae per ha) or high degree of uncertainty in population size/trends **(2)**	**Moderate (13)**
Rio Encantado Area	Some habitat protected, some at risk of being developed **(2)**	Connectivity **(3)**	Low **(3)**	Moderate **(2)**	Relatively low population size (0 to 5 imago and at less than 100 larvae per ha) or high degree of uncertainty in population size/trends **(2)**	**Moderate (12)**

Table 6-6 continued.

Population	Habitat Metrics				Population Metric	Resiliency
	Habitat Protection - Development Risk	Connectivity Habitat Fragmentation	Risk of Vegetation Clearing/Use of Pesticides (Low, Moderate or High)	Susceptibility to Stochastic Events (Low, Moderate or High)	Population Size/Trend Description	
Maricao Commonwealth Forest	Protected **(3)**	Both **(2)**	High **(1)**	High **(1)**	Relatively high population size (more than 20 imagoes and more than 500 larvae per ha) and/or growth **(6)**	**Moderate (13)**
Susua Commonwealth Forest	Protected **(3)**	Both **(2)**	High **(1)**	High **(1)**	Low population size with high degree of uncertainty in population trends **(2)**	**Moderately low (9)**

6.4.1 Future Resiliency

Future resiliency of three (3) of the five (5) PRHB populations is expected to decline to "Extirpated" under our "Most Likely" and "Worst Case" scenarios (Table 6-7). Collectively, these three (3) populations represent approximately 25 percent of the entire known PRHB population. The remaining two (2) populations (i.e., IQC and Maricao) are predicted to persist, but with lower levels of resiliency than currently. Only under the "Best Case" scenario will all five (5) populations persist at levels comparable to current conditions (Table 6-7).

Table 6-7. Summary of PRHB population resiliency under current and future scenarios.

Populations	Current (2020)	Worst Case	Most Likely	Best Case	Approximate percentage of total population[1]
IQC	Moderate (18)	Low (6)	Low (8)	Moderate (12)	53
Río Abajo	Moderate (15)	Extirpated	Extirpated	Moderate (13)	<5
Río Encantado	Moderate (14)	Extirpated	Extirpated	Moderate (12)	<5
Maricao	Moderately High (19)	Low (8)	Moderately Low (10)	Moderate (13)	21
Susúa	Low (11)	Extirpated	Extirpated	Moderately Low (9)	16

[1]Based on most recent (Barber 2018) field counts of imagoes (adult individuals).

6.4.2 Future Representation

According to our "Most Likely" and "Worst Case" scenarios, all areas and life zones which currently harbor PRHB populations are expected to become drier and warmer, with some (i.e., Río Abajo and Río Encantado) progressing from tropical moist forest to tropical dry forest (Figure 61). Under these scenarios, and with only 2 remaining populations (Table 6-7), the species would suffer a substantial decline in representation (as defined in Chapter 1).

6.4.3 Future Redundancy

Given the predicted extirpation of most (3/5) PRHB populations under our "Most Likely" and "Worst Case" scenarios, we expect an attendant and precipitous loss of population redundancy. Moreover, the only remaining populations (i.e., IQC and Maricao; Table 6-7) will most likely also become smaller, more fragmented, and subject to greater environmental stress.

6.5 Synthesis and Conclusions

Except for the Best Case scenario, the PRHB apparently faces significant reductions in Resiliency, Redundancy and Representation over the next 25 years. The overall threats to the species can be placed into two main categories: development and climate change. The continuing development – residential, commercial and tourist – both within and adjacent to areas currently occupied by PRHB will most likely increase over this time period, with attendant loss and degradation of suitable habitat, increased use of herbicides and pesticides, and greater risks of human-caused fires. These effects, both individually and collectively, have the potential to cause losses of not only annual reproductive cohorts, but also individual or multiple metapopulations, thereby further reducing species viability. Nevertheless, these adverse effects could potentially be lessened or mitigated by effective land use planning that considers PRHB biological and ecological needs and requirements. However, widespread and continuing lax application and enforcement of existing regulations that aim to protect PRHB habitat suggests efficacy of these measures will be limited in the future.

Although the adverse effects of development can potentially be lessened, the forecast changes in regional and local climate pose a much more daunting and irreversible challenge for the PRHB. The areas currently occupied by the species will most likely undergo increases in temperatures combined with a decrease in total precipitation over the next 25 years. Together, these changes will result in more severe and extensive drought conditions, while shifting some currently mesic life zones towards more xeric ones, further increasing risks of fires. The frequency of intense (Category 3-5) hurricanes will also increase over this time period. While the full ecological effects of these changes on the PRHB are yet unclear, it is likely that substantial changes in overall habitat and microhabitat (e.g., temperature, humidity) for a species whose ecology appears closely linked to specific current conditions (e.g., healthy *O. spinosa* populations) will have negative effects on the PRHB.

In summary, at the end of our predictive time horizon (year 2045) at least three (3) of the current five (5) PRHB populations will most likely have been extirpated, with those remaining (i.e., IQC and Maricao) incurring reductions in resiliency. Those predicted to be lost are the current populations at Río Abajo, Río Encantado, and Susúa, representing approximately 25 percent of the currently known total population size, which is already considered very small (less than 100 total individuals per population observed in any given year). Because of concomitant reductions in the remaining populations, the overall losses to the total PRHB population will be substantially greater than 25 percent, although impossible to accurately quantify at the current time.

LITERATURE CITED

Abel, J.R. and R. Deitz. 2014. The causes and Consequences of Puerto Rico's Declining Population. Federal Reserve Bank of New York. Current Issues in Economics and Finance, Vol 20. No 4. 8 pp. www.newyorkfed.org/reasearch/current_issues.

Barber, D. 2016. 2015-2016 Performance Report: "Rapid assessment of the Population Status of the Puerto Rican Harlequin Butterfly *(Atlantea tulita*) and threats to the species through its current distribution in Puerto Rico." FWS Award F15AC01220. 16 pp.

Barber, D. 2018. 2017-2018 Performance Report: "Rapid assessment of the Population Status of the Puerto Rican Harlequin Butterfly (*Atlantea tulita*) and threats to the species through its current distribution in Puerto Rico." USFWS Award #F15AC01220. 28 pp.

Barber, D. 2019. 2018-2019 Performance Report: "Rapid assessment of the Population Status of the Puerto Rican Harlequin Butterfly (*Atlantea tulita*) and threats to the species through its current distribution in Puerto Rico." USFWS Award #F15AC01220. 96 pp.

Biaggi-Caballero, J. 2009. Petition to list the Puerto Rican harlequin butterfly (*Atlantea tulita*) as an endangered species and to list the harlequin butterfly's critical habitat under the Endangered Species Act. 19 pp.

Biaggi-Caballero, J. and J. Lopez. 2010. Comments on 90-day finding on a petition to list the Harlequin butterfly as endangered. FWS-R4-ES-2010-0026. 12 pp.

Bond, W. J., Woodward, F. I. & Midgley, G. F., 2005. The global distribution of ecosystems in a world without fire. New Physiologist, 165:525–538.

Brandeis, T.J. and C.W. Woodall. 2008. Assessment of Forest Fuel Loading in Puerto Rico and the US Virgin Islands. Ambio Vol. 37, No. 7-8. pp. 557-562.

Carrión-Cabrera, J.E. 2003. "Estatus de Atlantea tulita (Dewitz, 1877) en Puerto Rico". A thesis submitted in partial fulfillment of the requirements for the degree of master in Science in Biology. University of Puerto Rico, Mayagüez Campus, Mayagüez, Puerto Rico. 76 pp.

Caribbean Landscape Conservation Cooperative. 2016, Puerto Rico Protected Areas Database [version of December, 20116] GIS data, San Juan, Puerto Rico. http://caribbeanlcc.org/interactive-map/

Carter, L. M., J.W. Jones, L. Berry, V. Burkett, J.F. Murley, J. Obeysekera, P.J. Schramm and D. Wear. 2014. Southeast and the Caribbean, Chapter 17. Climate Change Impacts in the United States: The Third National Climate Assessment, J.M. Melillo, T.C. Richmond and G.W. Yohe, Eds., U.S. Global Change Research Program, pp. 396-417. Doi:10.7930/JONP22CB. (http://nca2014.globalchange.gov/report/regions/southeast)

Castro-Prieto, J., M. Quiniones and G. William. 2016. Characterization of the network of protected areas in Puerto Rico. Caribbean Naturalist. 1-16.

Castro-Prieto, J.S. Martinuzzi, V.C. Radeloff, D.P. Helmers, M. Quiñones and W.A. Gould. 2017 (b). Declining human population but increasing residential development around protected areas in Puerto Rico. Biological Conservation 209. pp. 473-481.

Center for Disease Control and Prevention (CDC). 2017. Draft Programmatic Environmental Assessment Mosquito Control Activities Funded by CDC to Combat Zika Virus Transmission in the United States. CDC, Atlanta, GA 30329-4027. 21 pp.

Center for Disease Control and Prevention (CDC). 2017. https://www.cdc.gov/zika/vector/index.html, Accessed on March 11, 2019.

Chabert, J. 2015. Mariposa Arlequín (*Atlantea tulita*). Report on the species. Fideicomiso de Conservación de EL Pastillo. 5 pp.

Crandall, K.A., O.R. Bininda-Emonds, G.M. Mace, and R.K. Wayne. 2000. Considering evolutionary processes in conservation biology. *Trends in ecology & evolution*, *15*(7), pp. 290-295.

Department of Natural and Environmental Resources. 2004. Reglamento para regir las especies vulnerable y en peligro de extinción en el Estado Libre Asociado de Puerto Rico. Commonwealth of Puerto Rico, Reg. 6766. 61 pp.

Department of Natural and Environmental Resources. 2010. Designación de hábitat crítico para la mariposa endémica de Puerto Rico *Atlantea tulita*. Draft. San Juan , Puerto Rico. 26 pp.

Ewel J.J. and J.L. Whitmore 1973. The Ecological Life Zones of Puerto Rico and the U.S. Virgin Islands. Forest Service Research Paper ITF-18, 72 pp.

Feng, Y., R.I. Negron-Juarez, C. M. Patricola, W.D. Collins, M. Uriarte, J.S. Hall, N. Clinton, and J.Q. Chambers. 2018. Rapid remote sensing assessment of impacts from Hurricane Maria on forest of Puerto Rico. https://doi.org/10.7287/peerj.preprints.26597v1 | CC BY 4.0, 14 pp.

Franke, K.I. Karl, T.P. Centero, B. Feldmeyer, C. Lassek, V. Oostra, K. Riedel, M. Stanke, C.W. Wheat and K. Fischer. 2019. Effects of adult temperature on gene expression in a butterfly: identifying pathways associated with thermal acclimation. BMC Evolutionary Biology. https:// doi.org/10.1186/s12862-019-1363-y. pp. 1-16.

Furlan, E., J. Stoklosa, J. Griffiths, N. Gust, R. Ellis, R.M. Huggins and A.R. Weeks. 2012. Small population size and extremely low level of genetic diversity in island populations of the platypus, Ornithorhynchus anathus. Ecology and Evolution, pp. 844-857.

Glick, P., B.A. Stein, and N.A. Edelson, editors. 2011. Scanning the Conservation Horizon: A Guide to Climate Change Vulnerability Assessment. National Wildlife Federation, Washington, D.C.

Glista, D.J., T.L. DeVault, and J.A. DeWoody. 2007. Vertebrate road mortality predominantly impacts amphibians. Herp. Cons. and Biol. 3(1):77-87.

Gould, W., C. Alarcón, B. Fevold, M. Jiménez, S. Martinuzzi, G. Potts, M. Solórzano, and E. Ventosa. 2007. Puerto Rico Gap Analysis Project: Final Report. US Geological Survey, Moscow, ID, USA and the USDA Forest Service International Institute of Tropical Forestry, Río Piedras, PR, USA. 159 pp.

Hanski, I. 1999. Habitat Connectivity, Habitat Continuity, and Metapopulations in Dynamic Landscapes. Oikos, 87(2), 209-219. doi:10.2307/3546736

Hanski, I. and M. Gilpin, eds. 1997. Metapopulation Biology, Ecology, Genetics, and Evolution. Academic Press. London UK. 512 p.

Hanski, I., A. Moilanen, and M. Gyllenberg. 1996. Minimum Viable Metapopulation Size. The American Naturalist 147(4): 527-541. https://doi.org/10.1086/285864

Hanski, and M. Gilpin. 1991. Metapopulation dynamics: brief history and conceptual domain. Biological Journal of the Linnean Society, 42:3-16.

Henareh Khalyani, A., W. Gould, E. Harmsen, A. Terando, M. Quinones, and J. Collazo, 2016: Climate change implications for tropical islands: Interpolating and interpreting statistically downscaled GCM projections for management and planning. J. Appl. Meteor. Climatol. doi:10.1175/JAMC-D-15-0182.1. 55:265-282.

Hendry, A.P., M.T. Kinnison, M. Heino, T. Day, T.B. Smith, G. Fitt, C.T. Bergstrom, J. Oakeshott, P.S. Jørgensen, M.P. Zalucki, and G. Gilchrist. 2011. Evolutionary principles and their practical application. *Evolutionary Applications*, *4*(2), pp.159-183.

Herrera, D.A., T.R. Ault, J.T. Fasullo, S.J. Coats, C.M. Carillo, B.I. Cook and A.P. Williams. 2018. Exacerbation of the 2013-2016 Pan-Caribbean drought by anthropogenic warming. Geophysical Research Letters, 45. https://doi.org/10.1029/2018GL079408, pp. 1-8.

Higgins, L.G. 1958. Four new Melitaeinae genera (Nymphalidae). The Lepidopterists' News, 12(5-6): 161-164

Holdridge, L.R. 1947. Determination of World Plant Formations from Simple Climatic Data. Science Vol. 105, 2727:367-368.

Honnay, O. and Jacquemyn, H. 2007. Susceptibility of rare and common plant species to the genetic consequence of habitat fragmentation. Conservation Biology 21:824-831.

Hu, T. and R. Smith, 2018. The Impact of Hurricane Maria on the Vegetation of Dominica and Puerto Rico Using Multispectral Remote Sensing. Remote Sensing, 10(6), 827.

[IPCC] Intergovernmental Panel on Climate Change, 2007a: Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri, R.K and A. Reisinger, (eds.)]. IPCC, Geneva, Switzerland. 104 pp.

IPCC. 2018. Summary for Policymakers. In: Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty [V. Masson-Delmotte, P. Zhai, H. O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J. B. R. Matthews, Y. Chen, X. Zhou, M. I.Gomis, E. Lonnoy, T. Maycock, M. Tignor, T. Waterfield (eds.)]. World Meteorological Organization, Geneva, Switzerland.

Jennings, L.N., J. Douglas, E. Treasure and G.Gonzalez. 2014. Climate change effects in El Yunque National Forest, Puerto Rico, and the Caribbean region. Southern Research Station, USDA Forest Service, General Tech. Report SRS-193.

Kalarus K and P. Nowicki. 2015. How Do Landscape Structure, Management and Habitat Quality Drive the Colonization of Habitat Patches by the Dryad Butterfly (Lepidoptera: Satyrinae) in Fragmented Grassland? PLoS ONE 10(9):e0138557. https://doi.org/10.1371/journal.pone.0138557

Koda, K. and H. Nakamura. 2010. Effects of temperature on the development and survival of an endangered butterfly, *Shijimiaeoides divinus barine* (Leech) (Lepidoptera: Lycaenidae). Entomological Science 13, pp. 29-34.

Konvicka, M., K. Zimmermann, M. Klimova, V. Hula, and Z. Fric. 2011. Inverse link between density and dispersal distance in butterflies: field evidence from six co-occurring species. Population Ecology 54:91-101.

Lamas, G. 1989. The Neotropical butterflies described by Dewitz in 1877 (Lepidoptera, Rhopalocera). Entomofauna. ZEITSCHRIFT FüR Entomologue. ISSN 0250-4413. pp. 273-279.

Lankau R., P.S. Jorgensen, D.J. Harris, and A. Sih. 2011. Incorporating evolutionary principles into environmental management and policy. Evolutionary Applications, pp. 315-325.

Lugo, A. 2000. Effects and outcomes of Caribbean hurricanes in a climate change scenario. Science of the Total Environment 262. pp. 243-251.

Lugo, A. 2008. Visible and invisible effects of hurricanes on forest ecosystems: an international review. Austral Ecology 33, pp. 368-398.

Mateos, E., Santos, X. & Pujade–Villar, J., 2011. Taxonomic and Functional Responses to Fire and Post–Fire Management of a Mediterranean Hymenoptera Community. Environmental Management, 48:1000–1012.

Mathavan, S. and Pandian, T.J. 1975. Effect of temperature on food utilization in the monarch butterfly Danaus chrysippus. Oikos 26:60-64.

Mattila, A.L.K., A. Duplouy, M. Kirjokangas, R. Lehtonen, P. Rastask, and I. Hanski. 2012. High genetic load in an old isolated butterfly population. Proceedings of the National Academy of Sciences 109(37): E2496-E2505.

Mendez-Tejeda, R., M. Santos-Corrada, S. Ortiz-Morales, and O. Claudio-Vargas. 2015. Environmental and Economic Impact of Forest Fires in Puerto Rico 2013-2014. Scientific Research Publishing. Open Journal of Forestry 5. pp. 353-363. http://www.scirp.org/journal/ojf

Méndez-Lázaro, P.A., A. Nieves-Santiago, and J. Miranda-Bermúdez. 2014. Trends in total rainfall, heavy rain events, and number of dry days in San Juan, Puerto Rico, 1955-2009. Ecology and Society 19(2): 50. http://dx.doi.org/10.5751/ES-06464-190250

Miller, J.Y. 1994. Behavior in butterflies as a means of conservation: comparison of insular and continental fauna. Florida Entomologist 77(1). pp. 74-84.

Monzón-Carmona, O. 2007. "Influencia de la calidad de habitat, area y la conectividad sobre bod poblaciones de Atlantea tulita Dewitz (Lepidoptera: Nhymphalidae) en Quebradillas: su manejo y conservación . A thesis submitted in partial fulfillment of the requirements for the degree of master in Science in Biology. Universidad Metropolitana, San Juan, Puerto Rico. 88 pp.

Niitepõld, K. 2009. Genotype by temperatura interactions in the metabolic rate of the Glanville fritillary butterfly. Journal of Experimental Biology 213, pp. 1042-1048

[NOAA] National Oceanic and Atmospheric Administration. 2018. NOAA Historical Hurricane Tracks. https://oceanservice.noaa.gov/news/historical-hurricanes/ accessed 5/1/2018.

Nowicki, P. and V. Vrabec. 2011. Evidence for positive density-dependent emigration in butterfly metapopulations. Oecologia 167:657-665.

Pérez-Asso, A.R., J.A. Genaro and O.H. Garrido. 2009. Butterflies of Puerto Rico. Edit Cocuyo. ISBN 13: 978-0-9822388-0-6. 140 pp.

Puerto Rico Planning Board (PRPB). 2010. Borrador del Reglamento de Calificación Especial del Área de Planificación Especial Quebradillas-Isabela-Aguadilla (APEQIA). Published on December 20, 2010.

Puerto Rico Planning Board. Puerto Rico Interactivo. http://gis.jp.gobierno.pr/website/pri/ambiental/viewer.htm accessed on March 21, 2010.

Puerto Rico Climate Council (PRCCC). 2013. Puerto Rico's State of the Climate 2010-2013: Assessing Puerto Rico's Social-Ecological Vulnerabilities in a Changing Climate. Puerto Rico Coastal Zone Management Program, Department of Natural and Environmental Resources, NOAA Office of Ocean and Coastal Resource Management. San Juan, PR.

Pometto, S. 2014. Repair of the proboscis of brush-footed butterflies (Lepidopetera: Nymphalidae). A thesis presented to the Graduate School of Clemson University in partial fulfillment of the requirements for the degree of Master of Science Entomology. 106 pp.

Rawlins, J.E. and R.C. Lederhouse. 1981. Developmental Influences of Thermal Behavior on Monarch Caterpillar (*Danus plexippus*): an adaptation for migration (Lepidoptera: Nymphalidae: Danainae). Journal of Kansas Entomological Society, Vol, 54, No. 2, pp. 387-408.

Redford, K.H., G. Amato, J. Baillie, P. Beldomenico, E.L. Bennett, N. Clum, R. Cook, G. Fonseca, S. Hedges, F. Launay, S. Lieberman, G.M. Mace, A. Murayama, A. Putnam, J.G. Robinson, H. Rosenbaum, E.W. Sanderson, S.N. Stuart, P. Thomas, J. Thorbjarnarson. 2011. What does it mean to conserve a (vertebrate) species? BioScience 61:39-48.

Robbins, A.M., C-M Eckelman and M. Quiñones. 2008. Forest Fires in the Insular Caribbean. Ambio Vol 37. No 7-8. pp. 528-534.

Santiago-García, R.J., S. Molina-Colón, P. Sollins and S.J. Van Bloem. 2008. The Role of Nurse Trees in Mitigating Fire Effects on Tropical Dry Forest Restoration: A Case Study. Ambio Vol. 37No. 7-8. pp. 604-608.

Santiago, X.B., D. Rivera, A. Pabón, and A. Garcia. 2016. An Examination of the Use of Pesticides in Puerto Rican Agriculture. RURALS: Review of Undergraduate Research in Agricultural and Life Sciences. Vol 10 Issue I, September 11, 2016. 11 pp.

Serrat, A., Pons, P., Puig–Gironès, R. & Stefanescu, C., 2015. Environmental factors influencing butterfly abundance after a severe wildfire in Mediterranean vegetation. Animal Biodiversity and Conservation, 38.2: 207–220.

Shaffer, M.L. and B.A. Stein. 2000. Safeguarding our Precious Heritage. Precious Heritage; The Status of Biodiversity in the United States. B.A. Stein, L.S. Kutner and J.S. Adams (eds.). Oxford University Press pp. 301-321.

Sgro, C.M., A.J. Lowe, and A.A. Hoffmann. 2011. Building evolutionary resiliency for conserving biodiversity under climate change. Evolutionary Applications, 4(2), pp. 326-337.

Sutcliffe, O.L., C.D. Thomas and D. Moss. 1996. Spatial synchronomy and asynchronomy in butterfly population dynamics. Journal of Animal Ecology 65, pp. 85-95.

Thaxton, J.M., S.J. Van Bloem, and S. Whitmire. 2012. Fuel conditions associated with native and exotic grasses in a subtropical dry forest in Puerto Rico. Fire Ecology 8(3):9-17. doi: 10.4996/fireecology.0803009

USDA. 2008. The Puerto Rico Gap Analysis Project. Volume 1: Land Cover, Vertebrate Species Distribution, and Land Stewardship. International Institute of Tropical Forestry. General Techical Report IITF-GTR-39. pp. 20-21.

USGCRP, 2018: Impacts, Risks, and Adaptation in the United States: Fourth National Climate Assessment, Volume II [Reidmiller, D.R., C.W. Avery, D.R. Easterling, K.E.Kunkel, K.L.M. Lewis, T.K. Maycock, and B.C. Stewart (eds.)]. U.S. Global Change Research Program, Washington, DC, USA. doi: 10.7930/NCA4.2018.(https://nca2018.globalchange.gov/)

U.S. Fish and Wildlife Service (USFWS). 2018. Species status assessment report for the Dakota skipper (*Hesperia dacotae*). 97 pp.

Vandewoestijne, S., N. Schtickzelle, and M. Baguette. 2008. Positive correlation between genetic diversity and fitness in a large, well-connected metapopulation. BMC Biology 6:46-46.

Van Bloem, S.J., P.G. Murphy, A.E. Lugo, R. Ostertag, M. Rivera-Costa, I. Ruiz-Bernard, S. Molina-Colon, and M. Canals-Mora. 2005. The Influence of Hurricane Winds on Caribbean Dry Forest Structure and Nutrient Pools. Biotropica 37(4):571-583.

Vargas, J. 2019. Habitat Description used by the Puerto Rican Harlequin Butterfly (*Atlantea tulita*). Final Report. FWS Award *F15AC01220*, Fort Worth Zoo. 38 pp.

Wisconsin Department of Natural Resources, Field guide to rare Lepidoptera of bogs and barrens glossary, http://dnr.wi.gov/org/land/er/invertebrates/butterflies_moths/glossary.htm, accessed on August 13, 2009.

Wong, S.C., A. Oksanen, A.L.K. Mattila, R. Lehtonen, K. Niitepold, and I. Hanski. 2016. Effects of ambient and preceding temperature and metabolic genes on flight metabolism in the Glanville fritillary butterfly. Journal of Insect Physiology 85, pp. 23-31.

Zackay, A. 2007. Random Genetic Drift & Gene Fixation. (https://pdfs.semanticscholar.org/1dcb/03aa10e74b1fc3d2dd1db06762cc19ce3f5a.pdf)

Appendix 1. List of plant species observed in the Puerto Rican harlequin butterfly habitat (Barber 2018, p. 72)

Scientific Name	**Common Name**	**Family**
Oplonia spinosa	Prickly bush	Acanthaceae
Odontonema cuspidatum	Coral de Jardín	Acanthaceae
Justicia mirabiloides	West Indian water-willow	Acanthaceae
Bidens pilosa	Spanish needle	Asteraceae
Boureria suculenta	Pigeon-berry	Boraginaceae
Bursera simaruba	Turpentine-tree	Burseraceae
Byrsonimia spicata	Hogberry	Malpighiaceae
Calophylum calaba	Sant-maria	Calophyllaceae
Capparis flexuosa	Caper tree	Capparaceae
Cecropia peltata	trumpet tree	Cecropiaceae
Cecropia schreberiana	pumpwood	Cecropiaceae
Citharexylum fruticosum	spiny fiddlewood	Verbenaceae
Clusia minor	cupey de monte	Clusiaceae
Clusia rosea	Scotch attorney	Clusiaceae
Coccothrinax barbadensis	Puerto Rico silver palm	Arecaceae
Coccoloba costata	uvilla	Polygonaceae

Appendix 1 continued.

Scientific Name	**Common Name**	**Family**
Coccoloba microstachya	puckhout	Polygonaceae
Coccoloba pubescens	grandleaf seagrape	Polygonaceae
Coccoloba uvifera	seagrape	Polygonaceae
Commelina diffusa	climbing dayflower	Commelinaceae
Comocladia glabra	carrasco	Anacardiaceae
Conocarpus erectus	button mangrove	Combretaceae
Croton flavens	yellow balsam	Euphorbiaceae
Dendropanax arboreus	Angelica tree	Araliaceae
Distictis lactiflora	liana fragante	Bignoniaceae
Eugenia biflora	blackrodwood	Myrtaceae
Eugenia confusa	redberry stopper	Myrtaceae
Epatorium odoratum	no common name	Asteraceae
Erithalis fruticosa	blacktorch	Rubiaceae
Erithalis odorifera	no common name	Rubiaceae
Garcinia hessi	no common name	Clusiaceae
Guettarda pugens	roseta	Rubiaceae
Guettarda scabra	wild guave	Rubiaceae

Appendix 1 continued.

Scientific Name	**Common Name**	**Family**
Ilex nitida	Puerto Rico holly	Aquifoliaceae
Krugiodendron ferreum	ironwood	Rhamnaceae
Lantana camara	red-sage	Verbenaceae
Lantana involucrata	buttonsage	Verbenaceae
Leucaena leucocephala	white leadtree	Fabaceae
Neea buxifolia	saltwood	Nyctaginaceae
Neoregelia resinos	no common name	Bromeliaceae
Passiflora suberosa	corkystem	Passifloraceae
Pimenta recemosa var grisea	bay-rum-tree	Myrtaceae
Plumeria krugii	no common name	Apocynaceae
Poitea paucifolia	retama	Fabaceae
Poitea punicea	caracol illo	Fabaceae
Psidium amplexicaule	mountain guava	Myrtaceae
Randia aculeata	white indigo-berry	Rubiaceae
Rondeletia inermis	cordobancillo	Rubiaceae
Sideroxylon cubense	espejuelo	Sapotaceae

Appendix 1 continued.

Scientific Name	**Common Name**	**Family**
Staehytarpeta jamaicensis	no common name	Verbenaceae
Tabebuia haemantha	roble cimarron	Bignoniaceae
Tabebuia heterophylla	white cedar	Bignoniaceae
Tabebuia karsoana	no common name	Bignoniaceae
Terminalia catappa	tropical almond	Combretaceae
Thouinia striata	ceboruquillo	Sapindaceae
Vernonia albicaulis	no common name	Asteraceae

Appendix 2. List of plants identified as Puerto Rican harlequin butterfly nectar sources (Barber 2018, p. 71)

Scientific name	**Quebradillas**	**Maricao**	**Susúa**
Erithialis fructicosa	X		
Paulinia pinnata	X		
Justicia mirabiloides	X		
Oplonia spinosa	X	X	X
Coccoloba uvifera	X		
Bourerria succulenta	X		
Lantana camara	X		
Lantana involucrata	X	X	
Croton rigidus			X
Stachytarpheta jamaicensis	X		
Randia aculeata	X	X	X
Stigmaphyllon emarginatum		X	

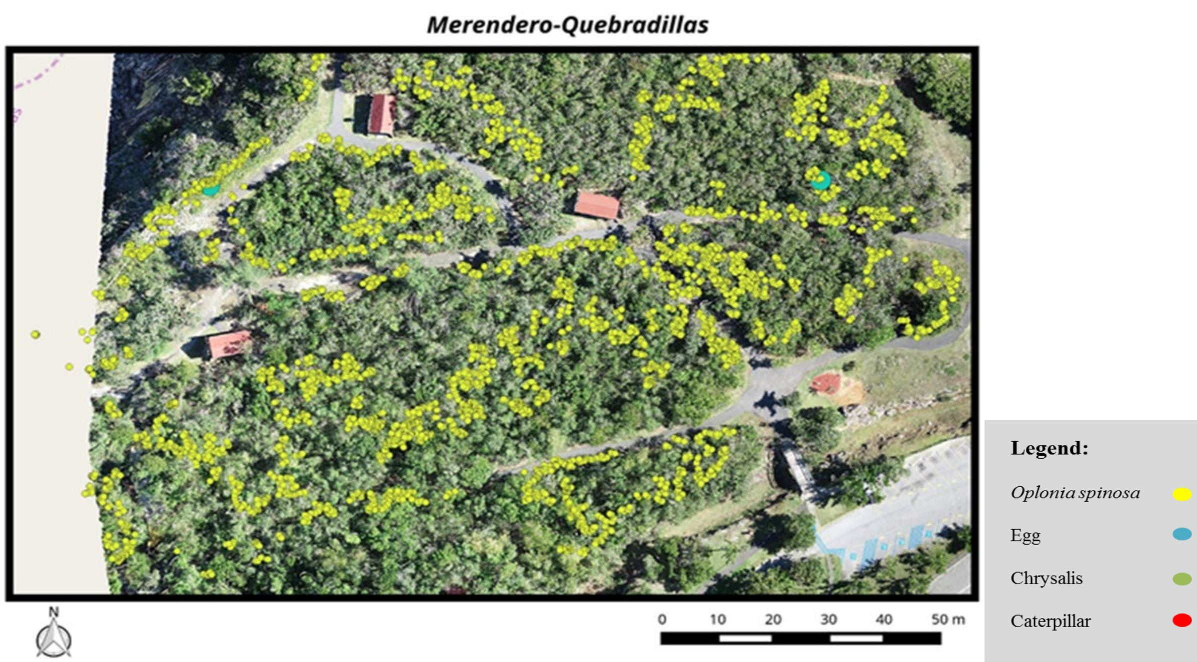
Appendix 2 continued.

Scientific name	**Quebradillas**	**Maricao**	**Susúa**
Vernonia albicaulis	X		
Tabebuia heterophylla	X	X	X
Poitea spp.	X	X	
Bidens urbanii		X	
Citharexylum fruticosum	X		
Guettarda ovalifolia		X	
Chromolaena sinuata (possibly)		X	

Appendix 3. Local Puerto Rican harlequin butterfly habitat descriptions and species occurrence records.

IQC Habitat

The Merendero in Quebradillas is the site where the PRHB has been most frequently observed in all of its life stages, which suggests the species is well adapted for the type of habitat at this site. There is a cliff at this site with no canopy coverage to the north and 50-70 percent canopy coverage to the south. *O. spinosa* is found in patches distributed along the northern cliff, and along the edges of the recreational trails and in some forested areas showing some level of disturbance in recent times (Vargas 2019, p. 2; Barber 2019, p. 37) (Map below). Here, *O. spinosa* has been observed mostly in the understory. The floral composition at this site, where there are around 29 plant species from 23 families, favors native species (See Appendix I). The most dominant tree species are the *Coccoloba uvifera* (16 percent), *Tabebuia heterophylla* and *Bursera simaruba* (13 percent each), and *Leucaena leucocephala* (12 percent), with a total of 41 percent of relative plant species abundance (Barber 2019, p. 37; Vargas 2019, p. 2). *Leucaena leucocephala* is the only non-native species that is apparently abundant in this site. The other three dominant species are native trees.



Map showing the *Oplonia spinosa* distribution and locations of the PRHB stages (if found during February survey) at El Merendero in Quebradillas (Vargas 2019, p. 3).

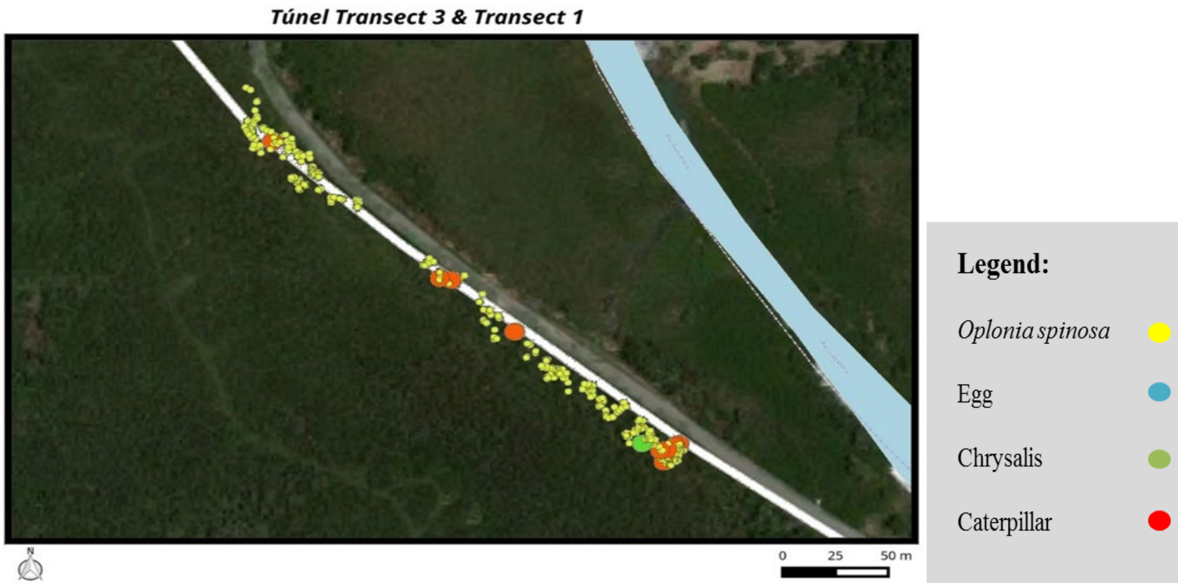
The Puente Blanco site is characterized by a cliff to the north with no canopy coverage and about 50-70 percent of canopy coverage to the south. The host plant *O. spinosa* is found in patchy distribution along the northern cliff, at the edges of a secondary road that provide access
Appendix 3 continued.

from Puente Blanco to Puerto Ermina, and in some forested areas showing some disturbance in recent times (Monzón 2007, p. 83) (Map below). Where we observed *O. spinosa*, it occupied most of the understory coverage.

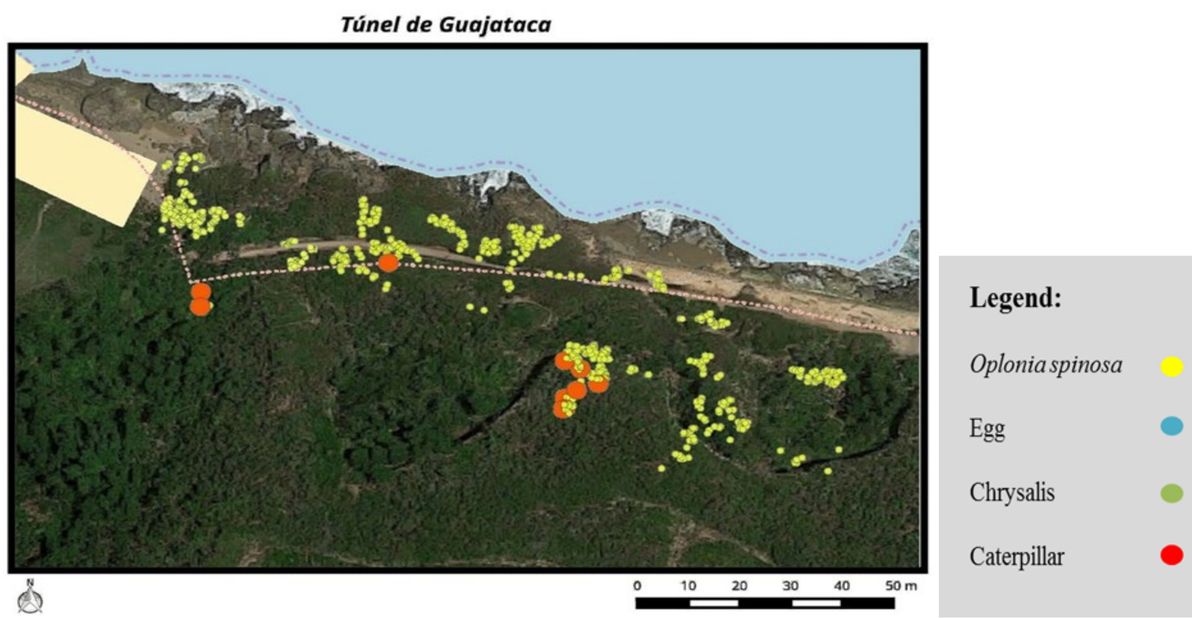
Map showing the *Oplonia spinosa* distribution at Puente Blanco in Quebradillas (Monzón 2007, p. 83).

At El Tunel de Guajataca area, the PRHB is often found along the road that provides access to the tunnel and the beach area, and along the former train rails that run at the base of the coastal cliff (Map below). Along the access road, the canopy cover is almost 100 percent starting at the bottom of the slope up to the beginning of the limestone hill (“mogote”). To the east there is a wetland, and the Guajataca River. The dominant species in this area are *Coccoloba uvifera, Ilex nitida, Bursera simaruba, Chrysophyllum oliviforme*, *Cecropia peltata* and *Clusia rosea*. *Olponia spinosa* also is found in two sections along the access road (Barber 2019, p. 13). At the former train rail, the habitat is characterized by limited canopy cover (less than 25 percent) with some dwarf vegetation close to the shore, and a limestone wall to the south (Map below). The dominant species are *Coccoloba uvifera, Oplonia spinosa, Terminalia catappa, Conocarpus erectus* and *Suriana maritima*. *Oplonia spinosa* dominates the open areas and under the *Coccoloba uvifera* where it has enough space to disperse.

Appendix 3 continued.



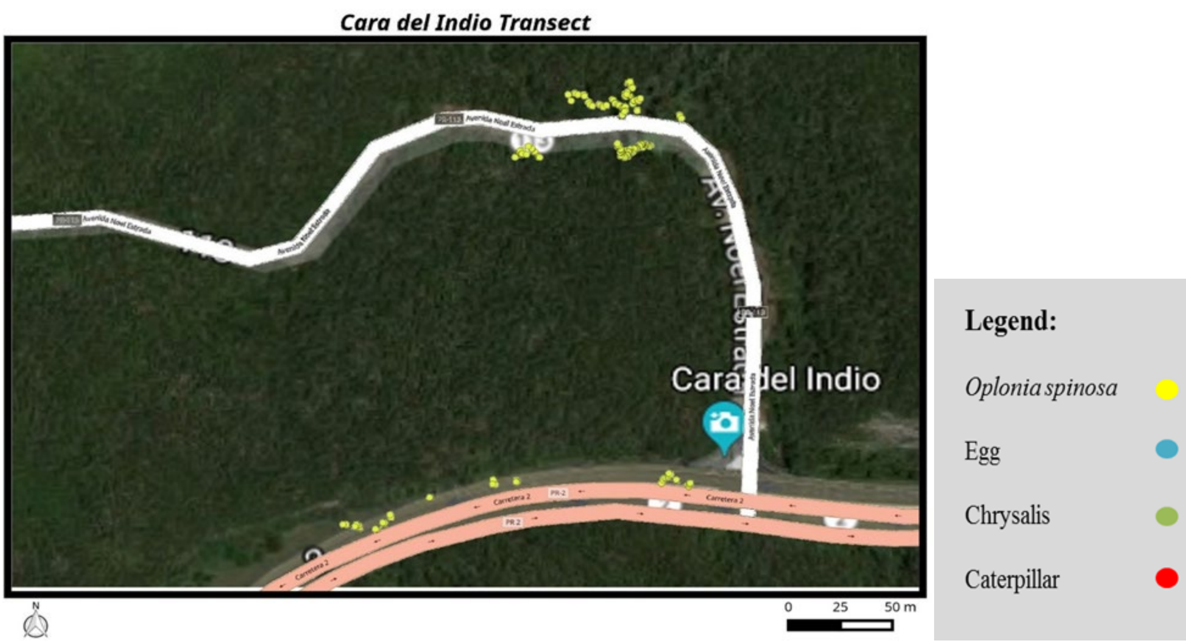
Map showing the *Oplonia spinosa* distribution and locations of the PRHB stages (if found during February survey) along the access road to El Tunel de Guajataca in Isabela (Vargas 2019, p. 4).



Map showing the *Oplonia spinosa* distribution and locations of the PRHB stages (if found during February survey) in the northern coastal cliff at El Tunel de Guajataca in Isabela (Vargas 2019, p. 5).

At Cara del Indio, the PRHB habitat is fragmented by Highway PR #2 (PR-2) on the south, State road PR-113 dissect the habitat on the north, and the Royal Isabela Resort also delimit the habitat Appendix 3 continued.

on the north. Along the segment of the road PR-133, the canopy cover is between 90 percent to 100 percent. *Oplonia spinosa* is found on the face of the limestone hill to the south of the road and at the edge of the cliff (Barber 2019. p. 6) (Map below). Dominant vegetation includes *Ilex nitida*, *Bursera simaruba*, *Commelina diffusa* and *Bidens pilosa*.



Map showing the *Oplonia spinosa* distribution and locations of the PRHB stages (if found during February survey) at Cara del Indio in Isabela (Barber 2019, p. 6).

At El Pastillo, the PRHB habitat is located on both sides of a secondary road that provides access to El Pastillo beach. The northern side of this road is dominated by *Leucaena leucocephala* and grasses, apparently as a result of habitat disturbances caused by former uses (e.g., cattle grazing) and vegetation removal. The southern side of the road has habitat with semi open areas (up to 75 percent of canopy cover) at the top of a small limestone hill. The dominant species in this area are *Terminalia catappa*, *Bursera simaruba*, *Leucaena leucocephala*, *Hylocereus trigonus*, *Ilex nitida* and some vines. *Oplonia spinosa* is found on both sides of the road, but is more abundant in the southern side (Barber 2019, p. 15) (Map below).

Appendix 3 continued.

Map showing the *Oplonia spinosa* distribution and locations of the PRHB stages (if found during February survey) at El Pastillo in Isabela (Barber 2019, p. 15).

Río Abajo Commonwealth Forest and Río Encantado Habitat

Both the Río Abajo Commonwealth Forest and the Río Encantado area, have very irregular topography, haystack hills with very steep slopes, subterranean water systems, caves, and natural depressions or sinkholes, all characteristic of the karst geological formations found along the northern karst belt of Puerto Rico. The majority of the vegetation in these areas is classified as subtropical moist forest, with a reduced representation of a subtropical wet forest at the Río Abajo Commonwealth Forest (Helmer et al 2002, p. 169; Morales and Estremera 2018, p. 1).

Much of the forest surrounding the Río Abajo Commonwealth Forest and the Río Encantado area is composed of secondary vegetation (DNR 1976, p. 126). The vegetation in these areas is more xerophytic than would be expected given the high amount of rainfall received in these areas (DNR 1976, p. 126). According to Morales and Estremera (2018, p. 2), the habitat where the PRHB occur in Río Encantado and Río Abajo Commonwealth Forest is mostly associated to mogotes habitat where the composition, physical structure, morphology and density of the vegetation change from as you move from the bottom to the top of the mogote. Tree species at the base of the mogotes are taller (canopy height average 10.3m (34 ft)) than at the top, where the vegetation is smaller expressing morphological features typically found in warmer and dryer conditions. Soil at the top is mostly shallow and rocky as compared to the soil at the base where it is deeper and moist. *Oplonia spinosa* is mostly found growing on the upper slopes of the mogotes where 90 degree steep walls rise abruptly all the way to the top (Morales and Estremera 2018, p. 2).

Appendix 3 continued.

Maricao Commonwealth Forest Habitat

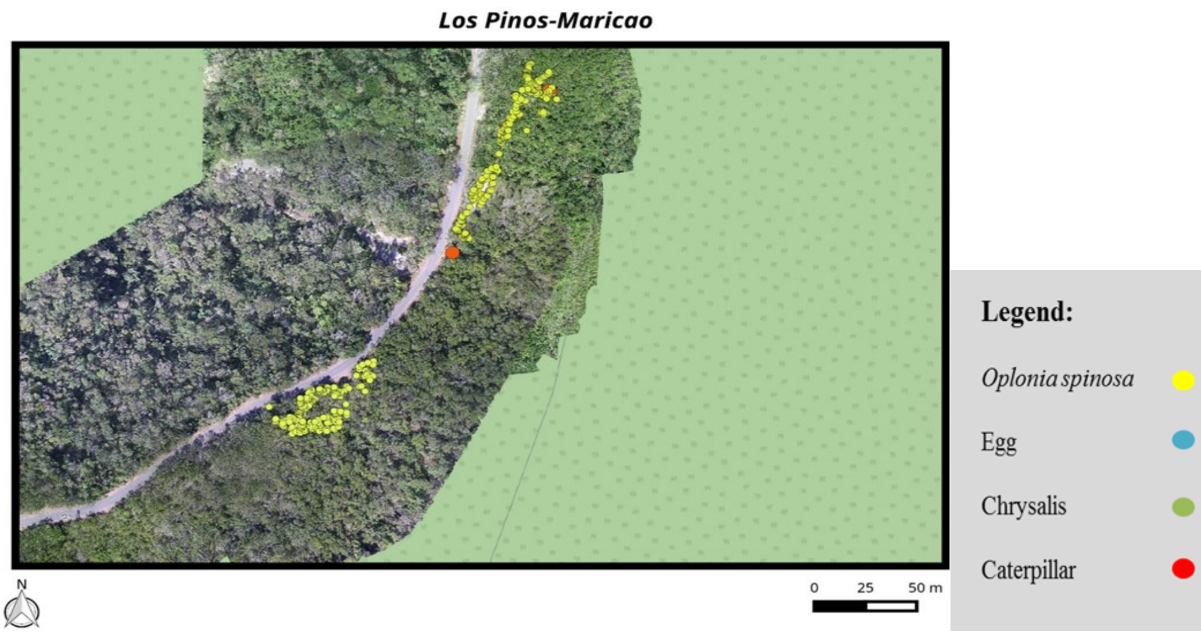
The Maricao Commonwealth Forest exemplifies vegetation types of serpentine soils, and probably has the most diversified flora of any area of the same size in Puerto Rico. The Forest is located on the west end of the Cordillera Central (central mountain range), and receives a mean annual precipitation of 2,500 mm (98.4 in; Ricart and Padrón 2010, p. 3; DNER 1976, p. 184). The rainfall ranges from 70 to 75 mm (2 to 3 in) during the month of January and February to approximately 350 mm (13.7 in) during the month of August, September and October (Ricart and Padrón 2010, p. 3). The mean monthly temperature varies from 20°C (68°F) during February to 23°C (73.4°F) during July, August and September, with a mean annual temperature of 21°C (69.8°F).

The PRHB is found in an area known as La Cantera, which is limited on the north by the State road PR-120, on the south by a steep cliff, on the west by an abandoned quarry, and on the east by Alto del Descanso trail. From road PR-120 to the Altos del Descanso the canopy cover ranges from 60 percent to less than 10 percent, but may reach up to 100 percent on the west of the Alto del Descanso trail. *Oplonia spinosa* is found from the edge of the road up to the top of the ridge, all in areas previously disturbed by the quarry activities at La Cantera (Barber 2019, p. 22) (Map below). In Alto del Descanso, the establishment of *O. spinosa* can be affected by the high understory and canopy vegetation cover.

Map showing the *Oplonia spinosa* distribution and locations of the PRHB stages (if found during February survey) at La Cantera in Altos del Descanso area, Maricao Commonwealth Forest (Barber 2019, p. 22).

Appendix 3 continued.

Los Pinos site is limited on the south by the State road PR 120 and on the north by the right of way of a Puerto Rico Energy and Power Authority (PREPA) distribution lines. In general, the canopy cover at this site fluctuates from 60 percent to 85 percent, with the exception of the areas along the road, trails, and underneath the power lines. *Oplonia spinosa* is found in 2 patches: one along a trail that provides access to the PREPA power line towers, and the other on a cliff to the north of the road (Barber 2019, p. 29) (Map below). Both patches are no more than 30m (98.4 ft) from each other.



Map showing the *Oplonia spinosa* distribution and locations of the PRHB stages (if found during February survey) at La Pinos, Maricao Commonwealth Forest (Barber 2019, p. 29).

Susúa Commonwealth Forest Habitat

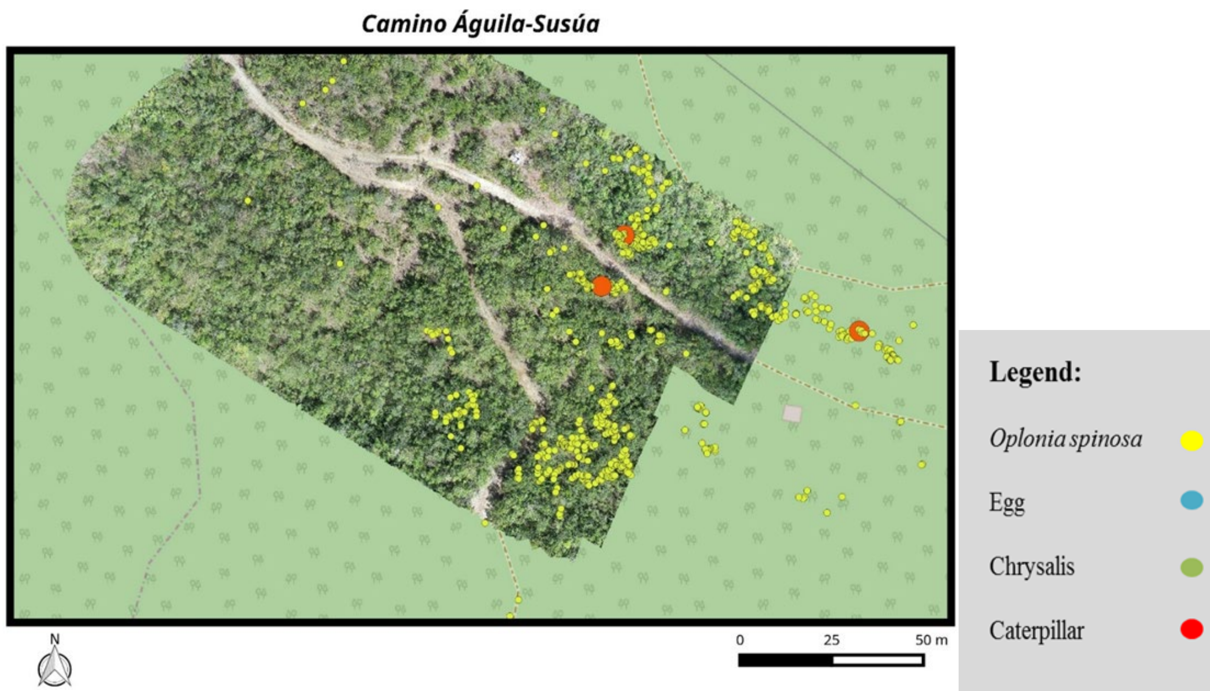
The Susúa Commonwealth Forest lies between the humid Cordillera Central and the dry coastal plains of the southern coast of Puerto Rico (DNER 1976, p. 224). Mean annual precipitation in this forest is 1,413 mm (55.6 in) and mean annual temperature is 23.9°C (75°F). Rainfall is generally heaviest in August, September and October and the driest season fall during February and March. The Susúa Commonwealth Forest represent not only the influence of a climatic transition zone (dry to moist), but also a combination of volcanic and serpentine soils. Over 90 percent of the Susua forest is classified as serpentine outcrop which consist of loses rocks scattered on the surface (DNER 1976, p. 224)

The Susúa Commonwealth Forest presents two vegetation associations: dry slope forest and gallery forest, falling in the sub-tropical dry/moist life zone on serpentine-derived soil (Helmer et

Appendix 3 continued.

al. 2002, p. 169). The serpentine-derived soil supports unique vegetation, which harbors several endemic species, but does not support any significant agriculture and timber production (DNR 1976, p. 224). Trees are slender, open crowned, and usually less than 12 m (40 ft) tall. The understory is open due to its excessively drained soil, which supports little herbaceous growth. The native vegetation has been significantly affected as a result of past land uses (e.g., harvest for charcoal) (DNR 1976, p. 224).

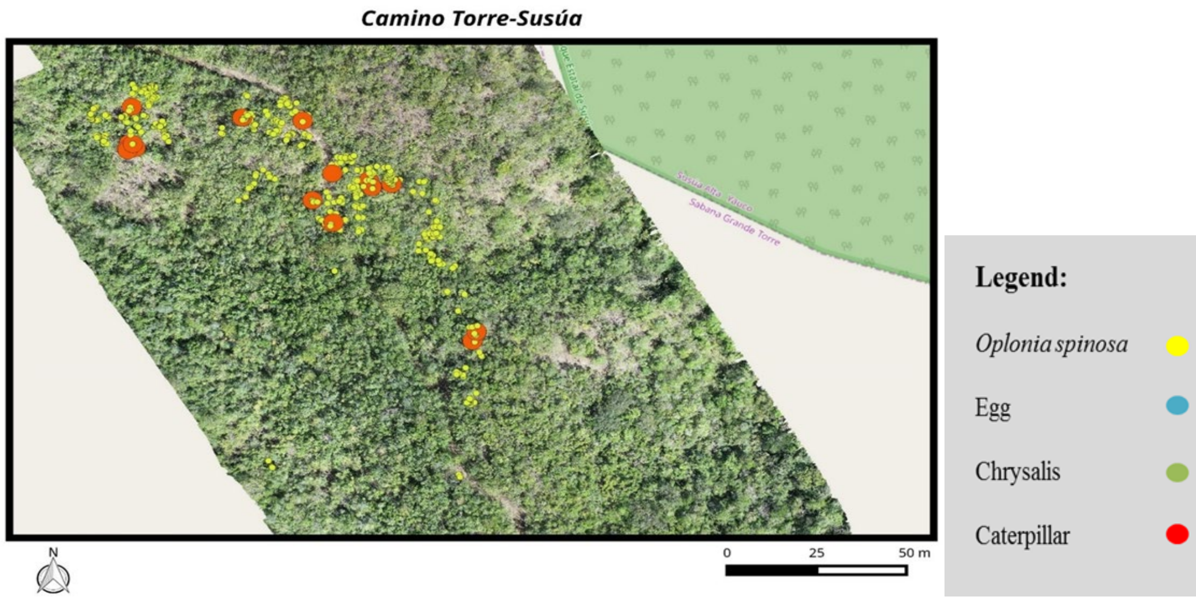
Within this forest, the PRHB occurs at Camino Torres (Torres trail) where the canopy cover ranges from 50 percent to 85 percent. Dominant species include: *Swietenia macrophylla, Thrinax morrisii, Tabebuia haemantha, Quadrella indica, Randia aculeata, Oplonia spinosa. Oplonia spinosa* is found in clusters outside the main trail in open areas or close to the ravines (Barber 2019, p.63) (Map below).



Map showing the *Oplonia spinosa* distribution at Torres trail, Susua Commonwealth Forest (Barber 2019, p. 63).

The PRHB also occurs in the Águila trail area where the canopy cover ranges between 10 percent to 85 percent (Barber 2019, p.64) (Map below). The species that dominate this landscape are *Oplonia spinosa*, *Tabebuia haemantha*, *Bursera simaruba*, *Thrinax morrisii*, *Swietenia macrophylla*, *Arthrostydium farctum*, *Garcinia hessii* and *Pimenta racemosa*.

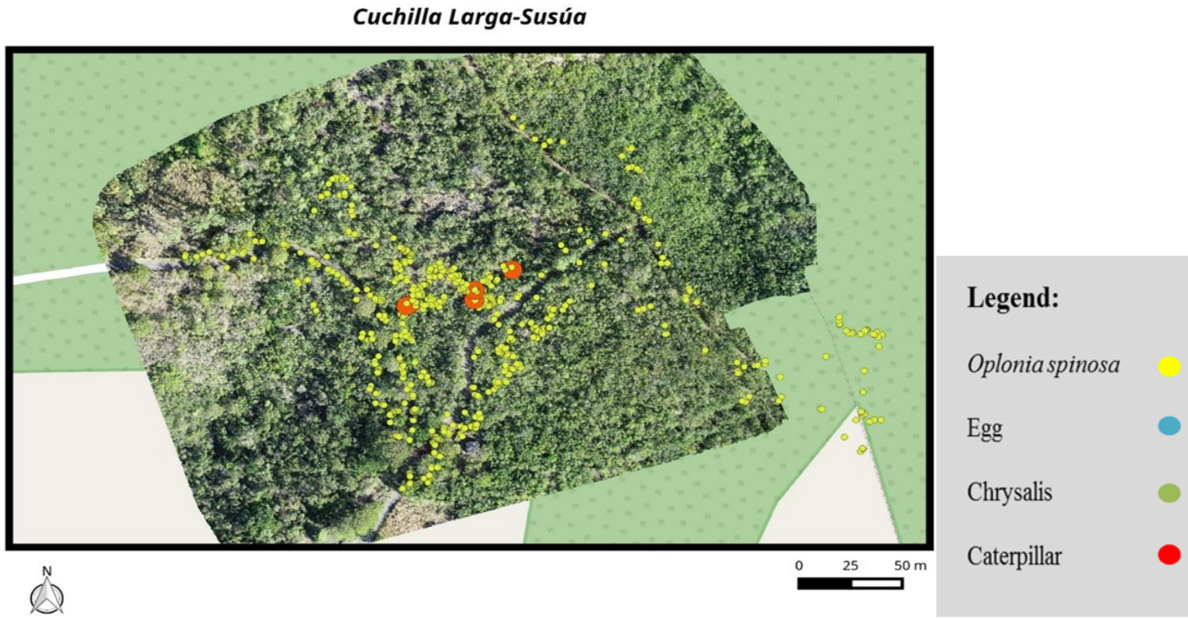
Appendix 3 continued.



Map showing the *Oplonia spinosa* distribution and locations of the PRHB stages (if found during February 2019 survey) at Camino Aguila, Susua Commonwealth Forest (Barber 2019, p.64).

The Cuchilla Larga is another PRHB site, located on the northwest section of the Susúa Commonwealth Forest, closer to the Maricao Commonwealth Forest (Map below). The canopy cover may vary between 50 percent to 85 percent. The dominant vegetation includes *Randia aculeata, Tabebuia* haemantha, *Chromolaena odorata, Clusia rosea, Swietenia macrophylla, Comocladia dodonaea, Pimenta racemosa, Neolaugeria resinosa, Bursera simaruba, Thrinax morrisii, Oplonia spinosa, Garcinia hessii,*and *Quadrella indica* (Vargas 2019, p.12).

Appendix 3 continued.



Map showing the *Oplonia spinosa* distribution and locations of the PRHB stages (if found during February 2019 survey) at Cuchilla Larga, Susúa Commonwealth Forest (Vargas 2019, p.11).