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

Version 1.6

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

April 2025

U.S. Fish and Wildlife Service
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VERSION UPDATES

Version 1.6 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 requests for formal peer review. Any peer review comments received will be considered and addressed, as appropriate, in an updated version to be labeled as Version 1.7.

Version 1.6 represent an updated to the number of Puerto Rican harlequin butterfly populations and to the species legal protection since it was listed. This document was prepared by Carlos Pacheco (U.S. Fish and Wildlife Service, Ecological Service- Region 4 Recovery Planning Team).

Version:	Date:	Prepared by:	Rationale:
1.5	April 2019	Carlos Pacheco and Tomas White	Inform listing process
1.6	February 2025	Carlos Pacheco	Inform recovery documents

EXECUTIVE SUMMARY

The Puerto Rican harlequin butterfly (*Atlantea tulita*), a species endemic to the island of Puerto Rico, whose range is limited to the Northern Karst physiographic region and the West-central Volcanic-serpentine physiographic region (Service 2019, p.15, 87 FR 73655). Throughout its range, we have identified six extant Puerto Rican harlequin butterfly populations, four found in the Northern karst region and two found in the West-central Volcanic-serpentine region. Each population is generally small, with less than 100 total individuals observed in any given year. Relative to historical conditions, the Puerto Rican harlequin butterfly distribution is now fragmented among discrete remnants of native forest located in four ecological life zones (or ecological settings) across two ecological regions, at elevations from 3 meters (m) (9 feet (ft)) to 867 m (2,845 ft) from sea level. 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., vegetation removal for grazing and haying), anthropogenic fires, pesticides, habitat restoration, public awareness and shifting climate conditions. An essential habitat features for the Puerto Rican harlequin butterfly 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.

We, the U.S. Fish and Wildlife Service (Service), updated the species status assessment (SSA) for the Puerto Rican harlequin butterfly, to inform the recovery planning documents: the recovery plan and the recovery implementation schedule. The previous SSA, ver. 1.5, was created to inform a new 12-month finding to determine whether the candidate species warrants listing (Service 2019, entire). The Puerto Rican harlequin butterfly was listed as threatened on January 3, 2023 (87 FR 73655), due to threats related to habitat modification and loss, due to its small populations size, and due to the results of the analyses of projected effects on the species caused by relevant factors (i.e., increasing on urban development rate, shifting climate conditions) that may negatively influence the continued existence of the Puerto Rican harlequin butterfly in a foreseeable future (Service 2019, entire).

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 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.

In this SSA, 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 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 one (1) Puerto Rican harlequin butterfly populations as having moderately high resiliency, four (4) as having moderately resiliency and one (1) as having 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 (Maricao Commonwealth Forest) occur in habitats managed for conservation that are surrounded by forest and have a low probability of being affected by human activities. The four populations classified as moderately (at Isabela, Quebradillas, and Camuy (IQC); Río Abajo Commonwealth Forest, Río Encantado Area and Guajataca Forest) occur in areas where human activities may negatively affect the species or have small population size. The population classified as low (Susúa Commonwealth Forest) occur in areas where human activities, stochastics events (hurricanes and wildfire), low population size and changes in climate conditions (severe drought and changes in temperature and humidity) may negatively affect the species. Currently, we consider resiliency at the species level (range wide) to be moderate.

To evaluate the future condition of the Puerto Rican harlequin butterfly, we placed the broad spectrum of factors that influence species' viability into two main categories: habitat modifications and changes in environmental conditions. 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 shifting climate conditions continue increasing at a moderate rate, with some decrease in population sizes (Most Likely Scenario); and (3) conditions whereby impacts from development and shifting climate condition continue increasing at a high rate and population sizes decreased substantially (Worst Case Scenario). Shifting climate conditions was an important factor in our analysis of Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly 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 shifts in climate conditions. 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 Puerto Rican harlequin butterfly, will most likely increase over this time frame, 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 Puerto Rican harlequin butterfly viability imposed by forecast changes to climate will be more challenging to address. While the full ecological effects of these changes on the Puerto Rican harlequin butterfly 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 prickly bush populations) will have negative effects on the Puerto Rican harlequin butterfly.

At the end of our predictive time horizon (year 2045) at least four (4) of the current five (5) Puerto Rican harlequin butterfly 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 Commonwealth Forest, Guajataca, Río Encantado area, and Susúa Commonwealth Forest, which represents approximately 66 percent of the currently known total population size. Because of related population reductions in the remaining populations, the overall losses to the total Puerto Rican harlequin butterfly population will be substantially greater than 66 percent, although impossible to accurately quantify at the current time.

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CHAPTER 1 – INTRODUCTION AND ANALYTICAL FRAMEWORK

1.1 Overview

The Puerto Rican harlequin butterfly (*Atlantea tulita*) is an endemic species to Puerto Rico, with a narrow range limited to the Northern Karst physiographic region and the West-central Volcanic-serpentine physiographic region of the Island. Throughout its range, six extant Puerto Rican harlequin butterfly populations have been identified: four found in the Northern karst region and two found in the West-central Volcanic-serpentine region (87 FR 73655). Each population is generally small, with fewer than 100 total adult individuals observed in any given year.

The Puerto Rican harlequin butterfly was listed as threatened on January 3, 2023 (87 FR 73655), due to threats related to habitat modification and loss, its small populations size, and the results of the analyses concerning projected effects on the species caused by relevant factors (i.e., increasing on urban development rate, shifting climate conditions) that may negatively influence the continued existence of the Puerto Rican harlequin butterfly in a foreseeable future.

In 2019, the U.S. Fish and Wildlife Service (Service), conducted a species status assessment (SSA) for the Puerto Rican harlequin butterfly to inform the 12-month finding to determine whether the candidate species warrants listing (Service 2019, entire). The 2019 SSA Report ver. 1.5 for the Puerto Rican harlequin butterfly describe the factors that have led to its status and those likely to influence its status into the future; assessed the health of individual populations given these influences; and describes the implications of predicted health and distribution on the 3Rs. The 2019 SSA served as the basis for the Service's decision on whether the species warrants protection under the Act.

Now, the Service is updating the 2019 SSA to inform the recovery planning documents: the Recovery Plan and Recovery Implementation Schedule (RIS). The updated version of the Puerto Rican harlequin butterfly SSA Report summarize relevant information that have become available since the 2019 SSA ver. 1.5 and the final rule for listing and critical habitat (87 FR 73655). It will also review the viability of the species based on the new information regarding the species' biology, distribution and threats and reassess the status of the resources and conditions needed to maintain its long-term viability.

1.2 Species Status Assessment Framework

The Species Status Assessment (SSA) framework (Service 2016, entire) is intended to guide 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 SSA is not a decisional document; rather, it provides a review of available information strictly related to the biological status of the Puerto Rican harlequin butterfly. Many species will have an SSA

developed during the listing process. However, for species that are currently listed, such as the Puerto Rican harlequin butterfly, an update on the SSA may be conducted during the recovery process. As such, the SSA report will be a living document. In this document, we consider what the species needs to maintain its viability by characterizing the status of the species in terms of its resiliency, redundancy, and representation (Wolf et al. 2015, entire).

For 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).

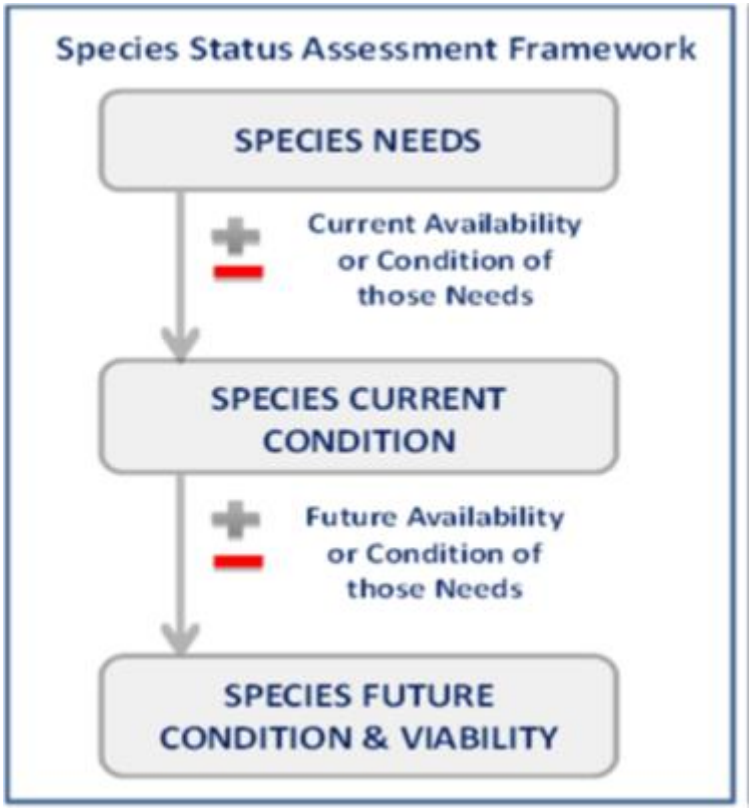


Figure 1-1. Species Status Assessment Framework

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. Populations need enough individuals, within habitat of adequate area and quality, to maintain survival and reproduction despite 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 Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly, we used the number of known populations to measure redundancy.

To evaluate the biological status of the Puerto Rican harlequin butterfly 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. The contents of this SSA Report provide an objective, scientific review of the available information related to the biological status of the Puerto Rican harlequin butterfly.

CHAPTER 2 – SPECIES INFORMATION

2.1. Taxonomy and Genetics

Atlantea tulita is a valid species belonging to the family Nymphalidae (http://www.nhm.ac.uk), the largest family of butterflies with more than 6,000 species distributed throughout most of the world. 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).

No genetic information for the Puerto Rican harlequin butterfly is available. No changes in nomenclature or taxonomic classification of the Puerto Rican harlequin butterfly since its listing as a separate and distinct species.

Currently, the genus *Atlantea* (Higgins 1958), is a genus endemic to the Caribbean and 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; 87 FR 73655).

The butterfly, *Atlantea tulita,* is an endemic species to Puerto Rico since the species has not found in another island in the Caribbean (87 FR 73655). This butterfly 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 this SSA, we refer to the common name as the Puerto Rican harlequin butterfly.

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

2.2. Species Description

The Puerto Rican harlequin butterfly is a medium size butterfly, when it reaches the adult stage has a wingspan of about 5.1 to 6 centimeters (cm) (2 to 2.5 inches (in)) wide. The species is member of the checker-spot butterfly group, is characterized by its orange, brownish-black and beige coloration patterns (Figures 2-2). The butterfly is brownish black at the thorax area with deep orange markings. Wings are largely brownish black with sub-marginal rows of deep orange spots and beige cells. The dorsal view of the forewings and the hindwings, 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. 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 species has no significant morphological differences between males and females (Figure 2-2). The male's abdomen is brownish black on the dorsal side and has orange and brown bands on the ventral side (Figure 2-3, left). The female’s abdomen is brownish black with white bands (Figure 2-3, right).

The Puerto Rican harlequin butterfly also belongs to the brush-footed butterfly and to the four-footed butterfly. Most of the butterflies belonging to the family Nymphalidae have greatly reduced their forelegs and stand on only four legs. The vestigial forelegs have a brush-like set of hairs that the butterfly uses only for touch and taste.

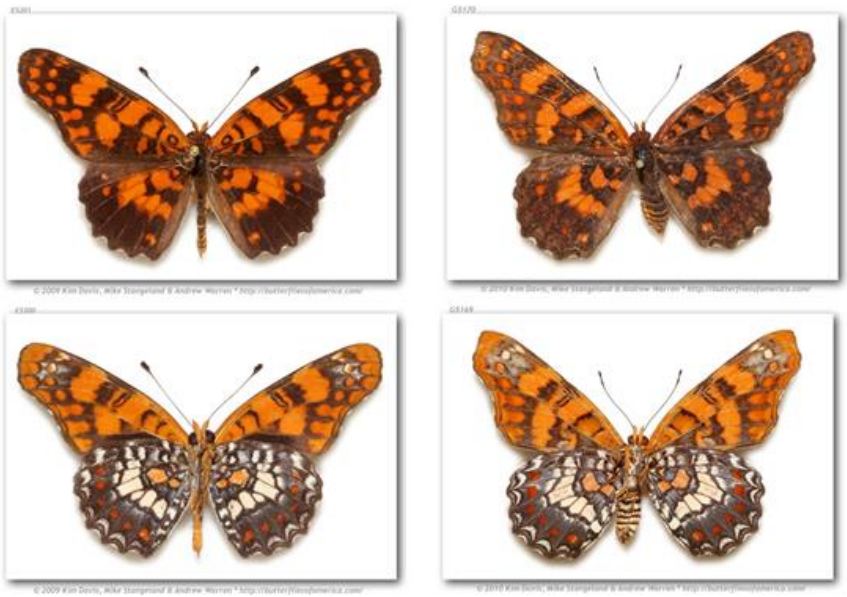


Figure 2-2. Photos showing the dorsal (top) and ventral (bottom) coloration patterns observed in the Puerto Rican harlequin butterfly. Male (left) and female (right). Photo downloaded from https://www.butterfliesofamerica.com.

Figure 2-3. Male (left) and female (right) of the Puerto Rican harlequin butterfly. 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 Puerto Rican harlequin butterfly has a complete life cycle including four distinct anatomical stages: egg, caterpillar, chrysalis, and imago (adult) (Figure 2-11). Their eggs are greenish oily spheres, with a yellowish incipient crown (Figure 2-6). The female lays the eggs in rows singly or in pairs, on the underside of tender twigs of the prickly bush. A female of the Puerto Rican harlequin butterfly 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). More than one female has been

documented laying eggs in the same branch and at the same time (Figure 2-3, Barber 2022, unpublished data, p.1).

Females may produce several broods in a single season (Biaggi-Caballero 2009, p. 2; 76 FR 31282, May 31, 2011, p. 31283, Service 2019, p.19). But the eggs viability (hatching success) rate have not been determined yet.

Figure 2-3. Left: female *Atlantea tulita* laying eggs on the host plant, prickly bush (*Oplonia spinosa*). Photo by José Vargas, 2018. Right: Two *Atlantea tulita* laying eggs on same branch of prickly bush branches. Photo by Diane Barber, 2022.

Figure 2-4. Photo (left) showing the yellowish crown on the eggs laid by *Atlantea tulita* on *Oplonia spinosa*. Photo (right): eggs of *Atlantea tulita* laid on the new growth (tender part) of prickly bush branches. Photo (left) by C. Pacheco, Service, 2011.

The Puerto Rican harlequin butterfly caterpillar (larva) goes through several size phases called instars (growth stage between molts) (Figure 2-8; DNER 2023, entire). When the eggs hatch (first instar), the caterpillar is less than 4.76 millimeters (mm) (0.19 in) long with a yellow body and black head. From the second to the sixth instar, the caterpillar 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 the anal plate (Figure 2-6). The body of the larva has spines with hairs on each body segment. The caterpillar reaches about 55.8 mm (2 in) long when it reaches its sixth instars

(i.e., fully grown caterpillar). The time from the first instar to reach the six instars is approximately 61 days (Figure 2-8.)

Figure 2-5. Photo (left and right) showing the first instars of the *Atlantea tulita*. Photo by C. Pacheco, Service, 2011.

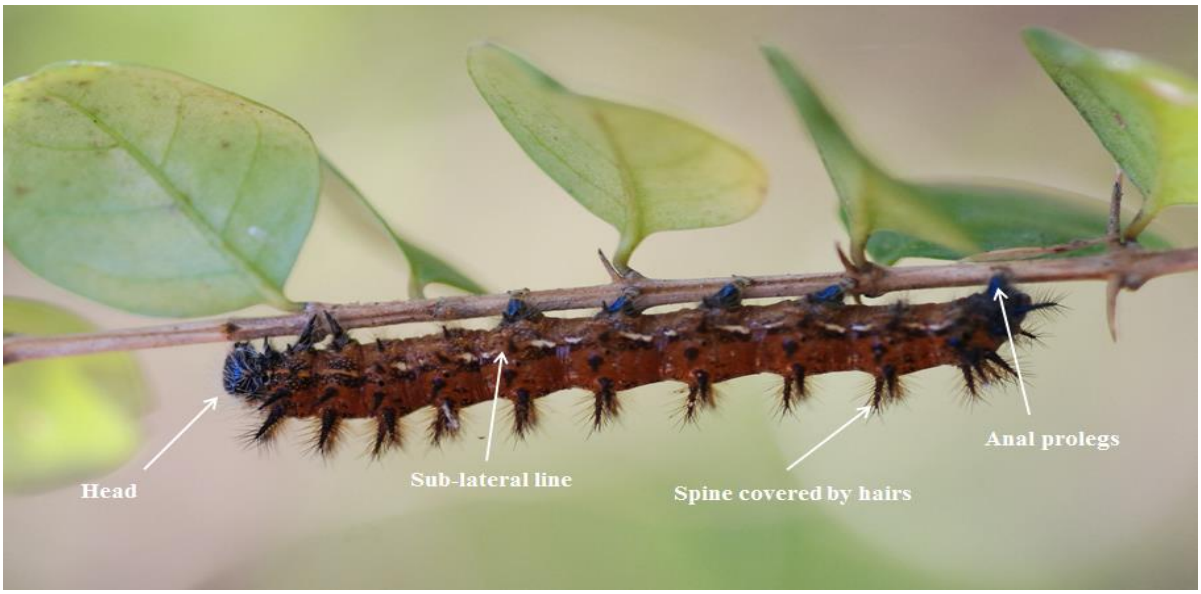


Figure 2-6. Caterpillar of the Puerto Rican harlequin butterfly. Photo by C. Pacheco, Service.

When the caterpillar is fully grown, it makes a button of silk that 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. The chrysalis (pupa from which the butterfly or imago, emerges) of the Puerto Rican harlequin butterfly is black, with orange and white dashes, and yellow pimples (Biaggi-Caballero 2009, p. 4) (Figure 2-7). Chrysalis size is around 3 cm (1.2 in). The species spends about 9 days finalizing its metamorphosis into a butterfly.

Figure 2-7. Chrysalis of the Puerto Rican harlequin butterfly. Photos by C. Pacheco, Service.

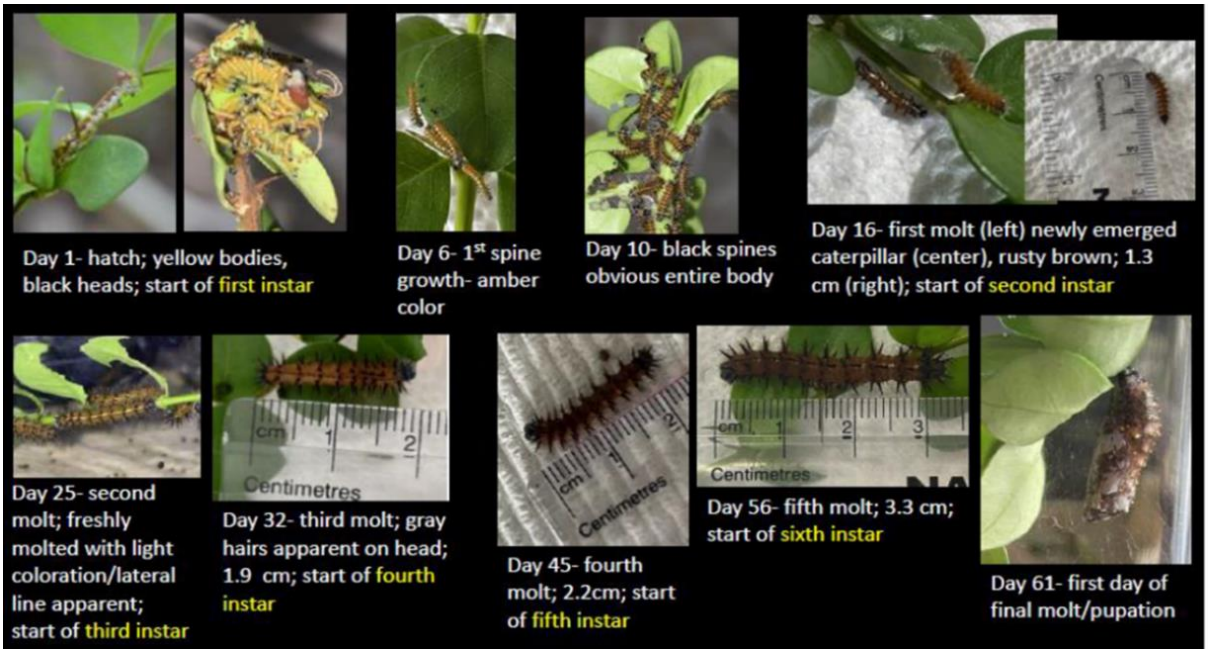


Figure 2-8. Showing information about sizes and days per each instar of the Puerto Rican harlequin butterfly (Barber and Bayo 2021, p.4; DNER, Fourth Technical Meeting Puerto Rican Harlequin Butterfly Working Group, October 7, 2023).

During its early life stages (i.e. egg and caterpillar), the butterfly uses exclusively prickly bush (*Oplonia spinosa*) as its host plant and food source (Figures 2-5 and 2-8). The Prickly bush belong to the Family Acanthaceae, a family of dicotyledonous flowering plants containing approximately 250 genera and about 2,500 species. Prickly bush is a common tropical shrub that is widely distributed in Puerto Rico (Figure 2-7). According to Lioger (1997, p. 42), prickly bush 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. New growth of prickly bush is observed a few days after rain events, being more abundant during the wet season (from April to November). The Puerto Rican harlequin butterfly 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).

Figure 2-9. Photo of the host plant Prickly bush (*Oplonia spinosa* (Jacq.) Rafinisque)), Family Acanthaceae; endemic to several Caribbean islands and widely distributed in Puerto Rico. Photos by C. Pacheco, Service, 2019.

In 2014, Andrés Vélez, a graduate student from the University of Puerto Rico, Mayagüez Campus, created a habitat model to predict the potential distribution of *Oplonia spinosa* in Puerto Rico (Vélez 2014, entire). This model was developed using herbarium collections of the species and indicated that *O. spinosa* may have a broad distribution across the island. Additionally, *O. spinosa* serves as the host plant for both the Dominican *Atlantea cryptida* and the Cuban *Atlantea perezi* (Biaggi-Caballero and Lopez, p. 3). While the host plant for the Jamaican *A. pantoni* is currently unknown, several other *Oplonia* species are present in the Jamaican karst, suggesting that these species may serve as potential host plants for the rare and elusive *A. pantoni*.

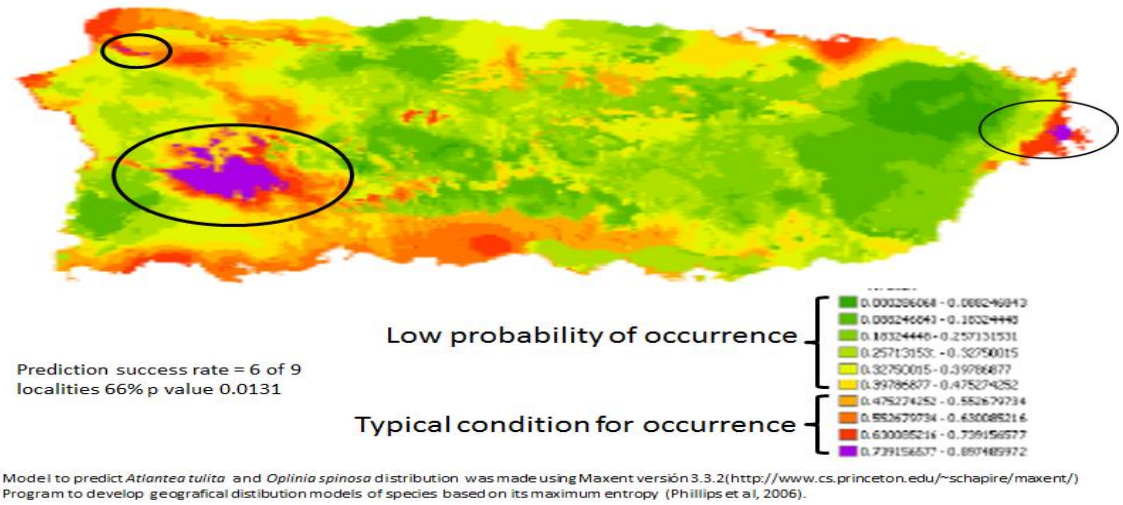


Figure 2-10. Map showing predicted areas with potential suitable habitat for *Oplonia spinosa* (Vélez 2014, entire).

2.3. Life History

Most of what is known about the Puerto Rican harlequin butterfly life history, demography and behavior come from field observations, information gathered from other species from the same family, and expert opinions.

-Life Cycle

As a member of the brush-footed butterflies, the Puerto Rican harlequin butterfly was believed to undergo five instars (molts) during its larval development and was believed that their life cycle from egg to imago in the wild can take around 125 days (Service 2019; 87 FR 73655). A recent study conducted by Fort Worth Zoo and the Puerto Rico Department of Natural and Environmental Resources reveals that in a controlled environment (laboratory environment) the life cycle of this butterfly has six instars and may takes around 70 days from egg to imago (Figure 2-8 and 2-11, Barber and Bayo 2021, p.4). Preliminary results suggests that the species' eggs take approximately 3 to 5 days to its eclosion (Barber and Bayo 2021, p.1). The time from the egg eclosion, passing through its 6 instars, and finalizing in its chrysalid, the species may take approximately 60 days (PRDNER 2023, entire). Study results also suggest that the species spent 8 to 10 days in its chrysalid stage until it finishes its metamorphoses into a butterfly (imago). However, there is a consensus among the species' experts that the length of the life cycle in the wild can be affected by factors such as temperature and humidity, particularly at the larval stage (Service 2019, 87 FR 73655, Fourth Technical Meeting Puerto Rican Harlequin Butterfly Working Group, October 7, 2023). Presently, Puerto Rican harlequin butterfly life expectancy in the wild is unknow.

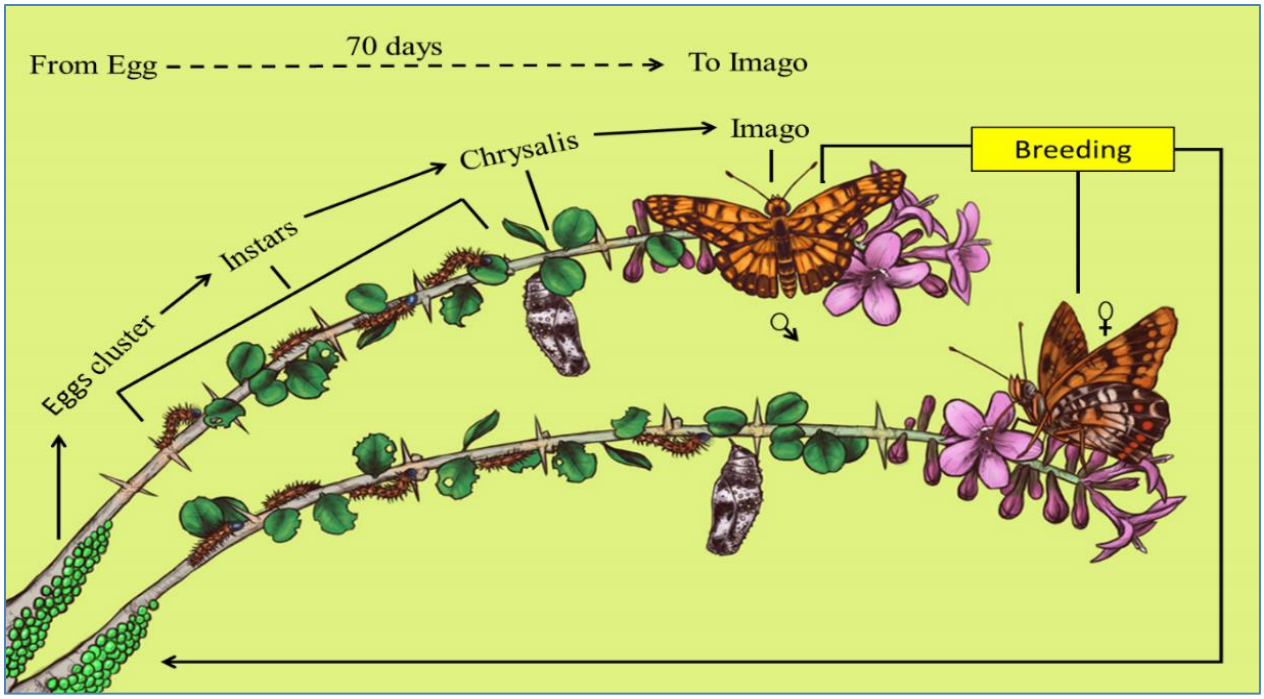


Figure 2-11. Conceptual diagram of the Puerto Rican harlequin life cycle.

-Movement/Dispersal

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) (3,366.1 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 monophagous habit of the first instar of the caterpillars, which feeds only on prickly bush (Carrión-Cabrera 2003, p. 40; Biaggi-Caballero 2009, p. 4).

-Mating Behavior

The Puerto Rican harlequin butterfly 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 2-9) or the ground, where mating occurs. Carrion-Cabrera (2003, p. 60) estimated the sex ratio of the Puerto Rican harlequin butterfly on 2.67 males per female. It is not well known if the Puerto Rican harlequin butterfly mates during a particular month of the year or year-round. However, data available reveal that all life stages of the Puerto Rican harlequin butterfly are observed year-round, suggesting that mating and oviposition may occur at any time during the year.

Figure 2-13. Photo of Puerto Rican Harlequin butterfly mating. Photo by José Chabert (President of "Fundanción EL Pastillo") at El Pastillo in the municipality Isabela.

After egg eclosion, the first instars devour the eggshells 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, Puerto Rican harlequin butterfly caterpillars crawl to the older and woody part of the host plant eating any new growth, including leaves and stems.

-Food Sources

Although the Puerto Rican harlequin butterfly is believed to be a specialist because of its monophagous habit of feeding only on prickly bush, Barber (2016, p. 9) documented a Puerto Rican harlequin butterfly larva feeding on *Odontonema cuspidatum* (commonly known in Puerto Rico as "coral de jardín") in Quebradillas (Figure 2-14). Like prickly bush, coral de jardín 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 Puerto Rican harlequin butterfly caterpillar also feeds on *Justicia mirabiloides* (commonly known as West Indian water-willow; or in Spanish as papayo montuno). The West-Indian water-willow, which is also in the family Acanthaceae, is a perennial herb native to Puerto Rico and the Virgin Islands. These rare observations of the Puerto Rican harlequin butterfly caterpillar feeding on plants other than prickly bush 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, prickly bush is an essential Puerto Rican harlequin butterfly food source.

Adults of the Puerto Rican harlequin butterfly 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 2-14. Caterpillars of *Atlantea tulita* feeding on *Oplonia spinosa* (prickly bush; left and center) and on *Odontonema cuspidatum* ("coral de jardín" right).

2.4. Resource Needs

The Puerto Rican harlequin butterfly needs the prickly bush for egg laying and for its larval feeding. In addition to prickly bush, the adult Puerto Rican harlequin butterflies need flowers of native trees as food sources. See Table 2-1. Water and nectar sources for adult Puerto Rican harlequin butterflies may vary according to the geographic area, life zone and habitat type. Temperature and relative humidity may be important for larval and adult survival. All the sites where the Puerto Rican harlequin butterfly occurs comprise a mosaic of forested habitat with canopy cover between 50 and 85 percent, average canopy height of 20 feet, forested corridor between suitable breeding sites (with the host plant covering more than 30 percent of the understory). Moreover, all sites have a close (within a 1 km radius) water source (e.g., creek, river, pond, among others).

As discussed above, individuals of the Puerto Rican harlequin butterfly have a variety of resource needs depending on life stage. These needs are summarized in Table 2-1 (below).

Table 2-1. Resources needed by the Puerto Rican harlequin butterfly to complete its life cycle (Service 2019, p. 22).

Resources needed by each Puerto Rican harlequin butterfly life stage		**Resource Function**	**Information Sources**
Eggs			
	Prickly bush (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: Prickly bush (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			
	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. SPECIES RANGE, DISTRIBUTION, POPULATION, HABITAT CONDITION AND SPECIES NEEDS.

3.1 Species Range

The Puerto Rican harlequin butterfly 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 in Quebradillas, and in Tallaboa, a location between the municipalities of Guayanilla and Peñuelas in the southern karst of the Island. 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 Puerto Rican harlequin butterfly 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, 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.

More recently, in 2018 and 2019, the Puerto Rican harlequin butterfly was found at three sites in the northern karst region: (1) in Río Encantado among the municipalities of Arecibo, Florida and Ciales: (2) at Río Abajo Commonwealth Forest between the municipalities of Arecibo and Utuado, and (3) at the Guajataca Forest in Isabela and Quebradillas (87 FR 73655). These new sites fall outside the historical range of the species in the northern karst, expanding its range through the municipalities of Isabella, Quebradillas, Camuy, Arecibo, Utuado, Florida and Ciales (Figure 3-1).

The Puerto Rican harlequin butterfly has been anecdotally reported (adults, but not other life stages) in other regions, including the municipalities of Aguadilla, San Sebastian, Barceloneta, Luquillo, Ceiba, Guánica, San Germán, Las Marias, and Lares (Rivera Declet 2015, p. 20). These anecdotal reports have not been confirmed (87 FR 73655).

Presently, the Puerto Rican harlequin butterfly is only known to occur in the northern karst region and in the west-central volcanic-serpentine region (Figure 3-2). Unfortunately, the fate of the Puerto Rican harlequin butterfly in the southern karst region is unknown because the species has not been found since 1926 (87 FR 73655). Therefore, the Puerto Rican harlequin butterfly is considered endemic to Puerto Rico whose range is limited to the northern karst physiographic region and the west-central volcanic-serpentine physiographic region in since it has not been found in other areas of the Island or in other islands in the Caribbean (87 FR 73655).

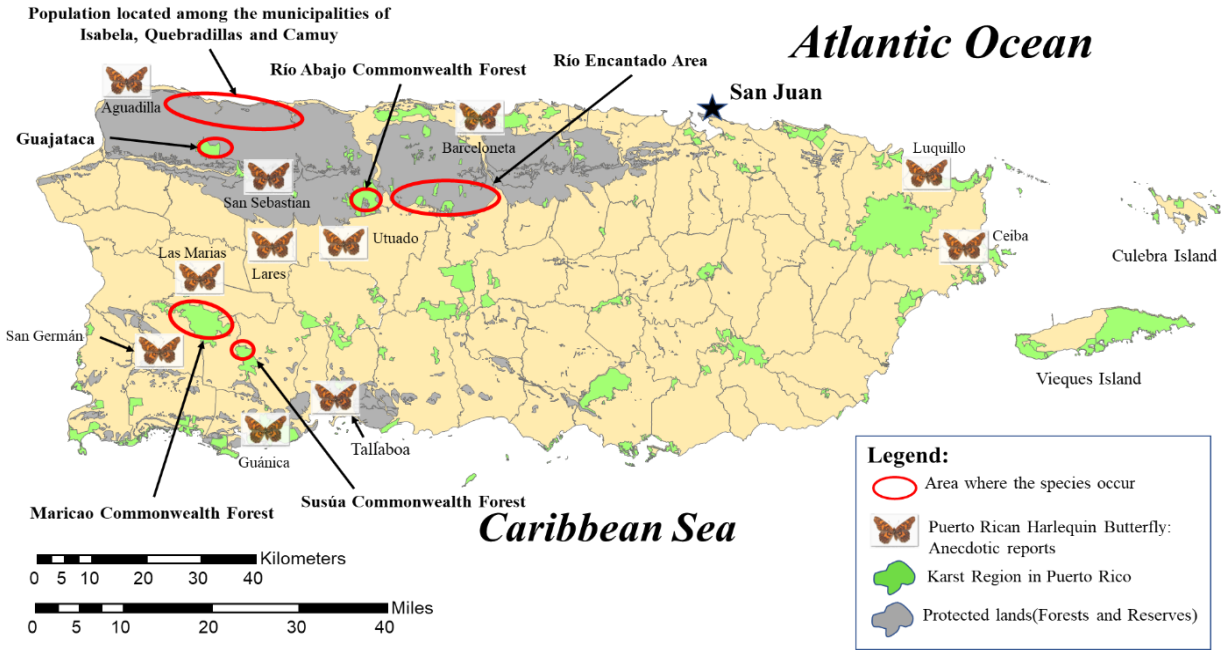


Figure 3-1. Map showing the areas where the Puerto Rican harlequin butterfly populations occur and anecdotal reports of the species in Puerto Rico.

3.2 Distribution and Population Structure

In this SSA we identified six areas currently occupied by the Puerto Rican harlequin butterfly that we refer to as a population, four in the northern karst region and two in the central-western volcanic-serpentine region (Figure 3-2). 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). Genetic data to determine true population structure are lacking. Conceptually, we treat each of the six populations as a metapopulation (Table 3-1, below), 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). The occurrence of the Puerto Rican harlequin butterfly in multiple sites (subpopulations) located at distance closer than 1 kilometer (0.6 miles) from each other support our rational for considering these occupied areas by the species as a metapopulation as the butterfly may move from one subpopulation to another. The key process in metapopulation dynamic are extirpation and recolonization of subpopulations, each at some rate over time (Hanski and Gilpin 1991, p. 8-9). Recolonization of extirpated subpopulations is dependent on the movement of individuals between subpopulations (immigration, emigration), such that temporarily unoccupied habitat patches exist and are important in overall metapopulation viability, particularly when suitable habitat on the landscape is dynamic.

Available information indicates that the Puerto Rican harlequin butterfly metapopulations occupy a small geographic area harboring remanent of native forest and are physically separated from other metapopulations by natural and manmade barriers (e.g., grass lands, agriculture lands,

highways, urban developed areas). Thus, 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 Puerto Rican harlequin butterfly, suggests there is little to no interaction between the metapopulations.

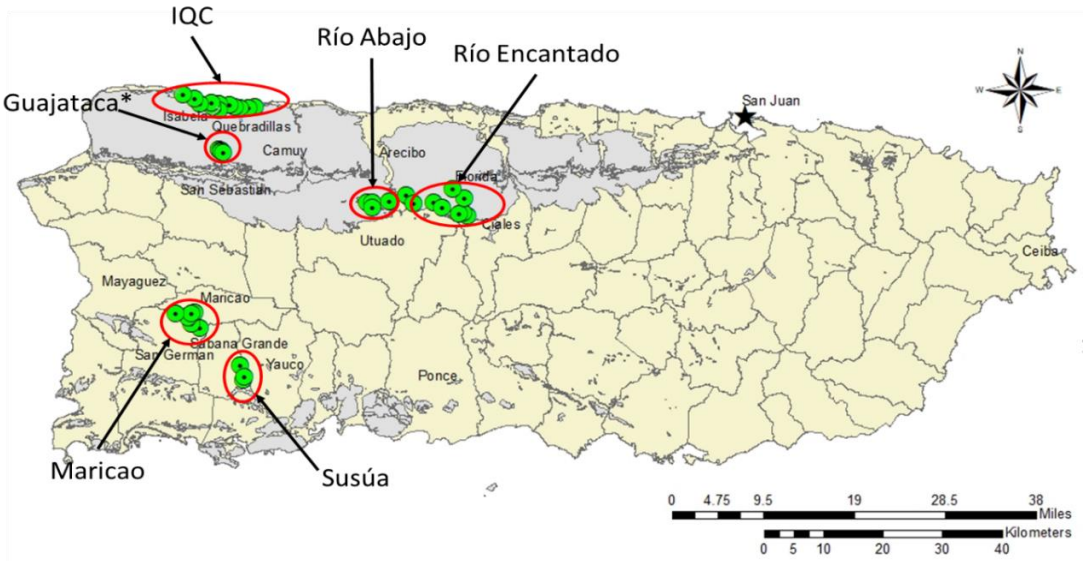


Figure 3.2. Map showing the current distribution and populations (red circles) of the Puerto Rican harlequin butterfly in Puerto Rico. Green dots are locations where the host plant *Oplonia spinosa* and more than one stage (egg, caterpillar, chrysalis, or imago) are frequently observed.

Northern Karst Region Metapopulations

In the northern karst region of Puerto Rico, the Puerto Rican harlequin butterfly is known to occur in four areas (Figure 3-2): along a coastal cliff in the municipalities of Isabella, Quebradillas, and Camuy; in the area of Río Encantado in the municipality of Florida, Ciales and Arecibo; in the Río Abajo Commonwealth Forest, between the municipalities of Arecibo and Utuado; and at the Guajataca Forest in the southern section of the municipality of Isabela and Quebradillas (87 FR 73655).

Isabela, Quebradillas and Camuy Metapopulation

In the area of Isabela, Quebradillas, and Camuy (IQC), the Puerto Rican harlequin butterfly is known to occur in the forested habitat along the coastal cliff that extend from 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. Within this area, the species is found often in 13 sites: six (6) sites in Isabella (e.g., in Guayabos' Sector, Royal Isabela, Los Milagros' Sector, El Pastillo, and Cara del Indio), six (6) sites in Quebradillas (e.g., in El Tunel de Guajataca, El Tunel Negro, El Merendero,

Terranova's cliff, Puente Blanco, San José's cliff, and Puerto Hermina) and one (1) site in Camuy (e.g., in Yeguadas' cliff). In those 13 sites, 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 3-3; Table 3.1). Additional sightings of the Puerto Rican harlequin butterfly imagoes have been anecdotally reported in other areas in the municipalities of Isabela, Quebradillas and Camuy, but the occurrence of the species was not confirmed at the time we finished this SSA report.

In the municipality of Isabela, the Puerto Rican harlequin butterfly currently occurs in six areas scattered along the forested cliff that extends from the Villa Pesquera at Coto Ward through the Royal Isabela Golf Course and El Pastillo, to Cara del Indio and El Tunel de Guajataca. Within these areas, all stages of the butterfly and the host plant have been observed in 46 sites (Service 2019, p. 28).

In the municipality of Quebradillas, the Puerto Rican harlequin butterfly currently occurs in five sites scattered along the coastal forested cliff that extends from El Merendero in Terranova ward through Puente Blanco, to Puerto Hermina in San José Ward. Additionally, the butterfly is found in the forested areas located on both sides along of "Calle Panoramica", a road that connects Puente Blanco and Puerto Hermina. The Puerto Rican harlequin butterfly also currently occurs farther inland in Quebradillas at Tunel Negro. Within this range, all life stages of the species and its host plant have been observed in 55 sites (Service 2019, p.27).

Furthermore, in the municipality of Camuy, the species 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 (Service 2019, p. 28).

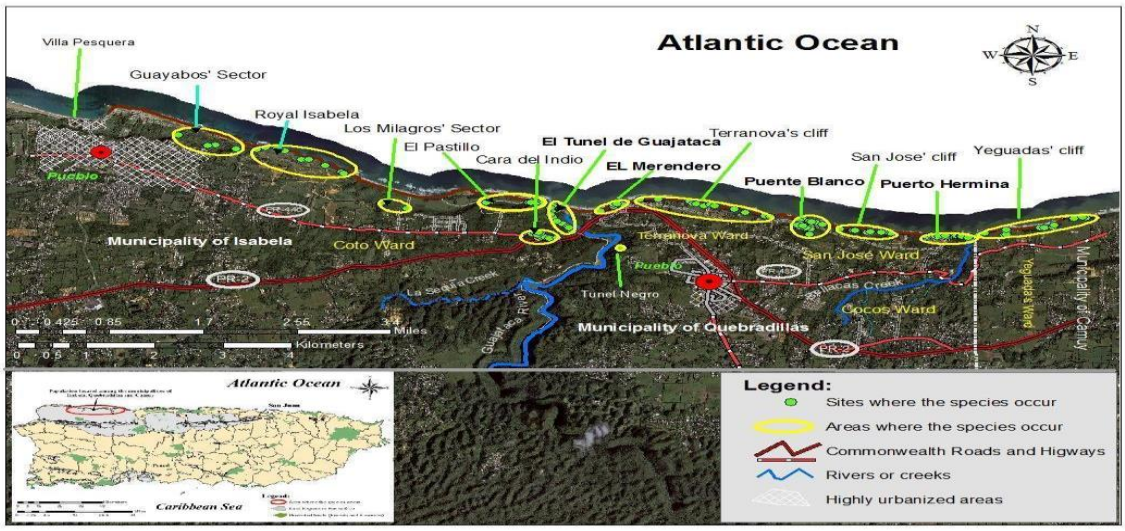


Figure 3-3. Map showing the distribution of the Puerto Rican harlequin butterfly along the municipalities of Isabela, Quebradillas and Camuy.

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 Puerto Rican harlequin butterfly is known to occur in three (3) areas scattered through the Río Encantado (Figure 3-4; Morales and Estremera 2018, unpublished data, p. 1; Service 2019, p. 28). Within these three areas, all life stages of the species and the host plant have been observed in 8 sites. In addition, imagoes of the Puerto Rican harlequin butterfly have been sighted in other forested areas adjacent to Frontón ward in Río Encantado (Morales and Estremera, Liga Ecológica Quebradillana, 2018, unpublished data, entire).

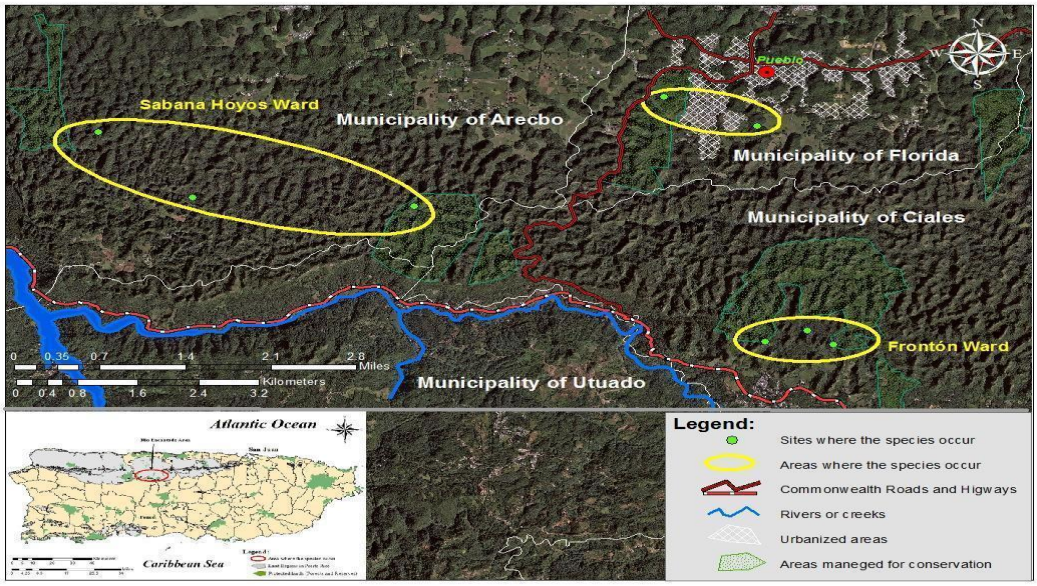


Figure 3-4. Map showing the distribution of the Puerto Rican harlequin butterfly in the Río Encantado area.

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, p.1), and is located approximately 29.9 km (20 mi) southeast of the Quebradillas' Puerto Rican harlequin butterfly population. Within the boundaries of the Río Abajo Commonwealth Forest, the species occurs in 3 sites, one adjacent to the west of State Road PR-10 and other two close to

Campamento Radley (Figure 3-5). These sites are in El Jobo Ward in Arecibo and located within a 1 km from each other (Service 2019, p. 29). In addition, sightings of imagoes of the species have been reported from other areas in Río Abajo Commonwealth Forest (Service 2019, p. 29; Ríos 2019, entire).

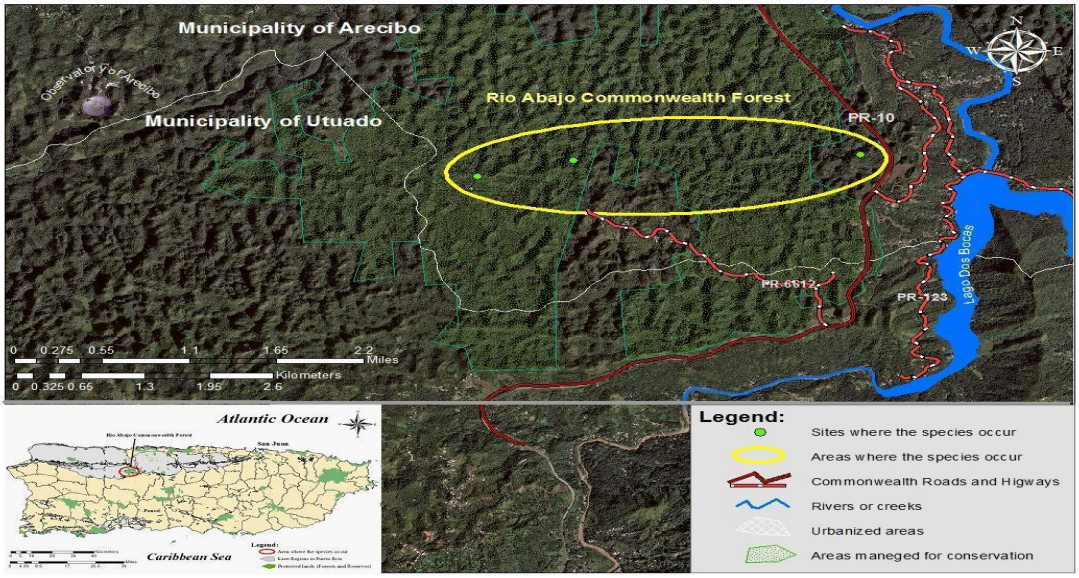


Figure 3-5. Map showing the distribution of the Puerto Rican harlequin butterfly at the Río Abajo Commonwealth Forest.

Guajataca Metapopulation

Guajataca Forest is located south of PR 2, between the municipalities of Isabela and Quebradillas. The Puerto Rican harlequin butterfly was first discovered in this area in July 2019 by José Román (PRDNER, Manager of the Guajataca Commonwealth Forest) (Rodríguez and Román 2019, entire). This new record of the Puerto Rican harlequin butterfly was situated on a private land approximately 4.9 miles (8km) south of the IQC metapopulation and about 0.60 miles (.97 km) west of the Guajataca River, in the municipality of Isabela. Currently, all life stages of the Puerto Rican harlequin butterfly are often found in 10 sites: six sites located along the La Caballa Trail (Rodríguez and Román 2019, entire) and four sites on a private land located 0.87 miles (1.39 km) northeast of La Caballa Trail and at approximately 0.37 miles (.59 km) east of the Guajataca River, in the municipality of Quebradillas (Figure 3-6; Morales and Monsón 2020, entire). Since 2019, sightings of imagoes of the species have been anecdotally reported in other areas of the Guajataca Commonwealth Forest (José Román, PRDNER, Manager of the Guajataca Commonwealth Forest, 2023, personal communication). Moreover, the occurrences and sightings of the Puerto Rican harlequin butterfly in Guajataca Forest have fallen within the boundaries of the designated critical habitat for the species (Figure 3-6, 87 FR 73655).

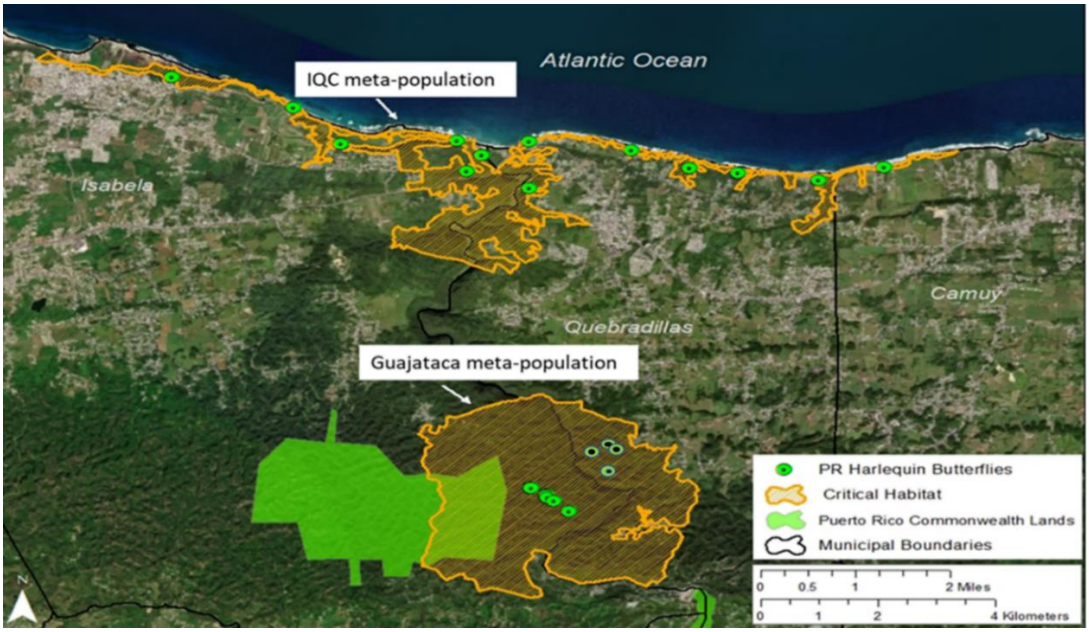


Figure 3-6. Map showing the distribution of the Puerto Rican harlequin butterfly at the Guajataca and IQC (Service 2023, unpublished data).

West-central Volcanic-serpentine Region

In the west-central volcanic-serpentine region, the Puerto Rican harlequin butterfly occurs in the Maricao and Susúa Commonwealth Forests, both are public forests managed for conservation by the Puerto Rico Department of Natural and Environmental Resources.

Maricao Commonwealth Forest Metapopulation

The Maricao Commonwealth Forest is in west-central Puerto Rico, encompassing 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). Within the Maricao Commonwealth Forest, all life stages of the Puerto Rican harlequin butterfly have been observed at seven sites: six of these sites are situated near Sate Road PR-120, while one is located near State Road PR-119 (Figure 3-7). The six sites near PR 120 are located between the km 16.0 (mi 9.9) and km 16.8 (mi 10.4) markers, all within a 1 km range of each other (Service 2019, p.30). In 2019, two new sites occupied by the species were discovered in the Maricao Commonwealth Forest: one site was located near State Road PR 119 on the “Alto del Descanso” trail in the western section of the forest, and the other was found on the Rosario River basin, approximately 0.60 m (0.37 mi) from State Road PR-120 (Barber 2019, p. 2; Service 2019, unpublished data, entire). The habitat between these subpopulations is moderate disturbed, characterized by forested areas fragmented by roads and trails.

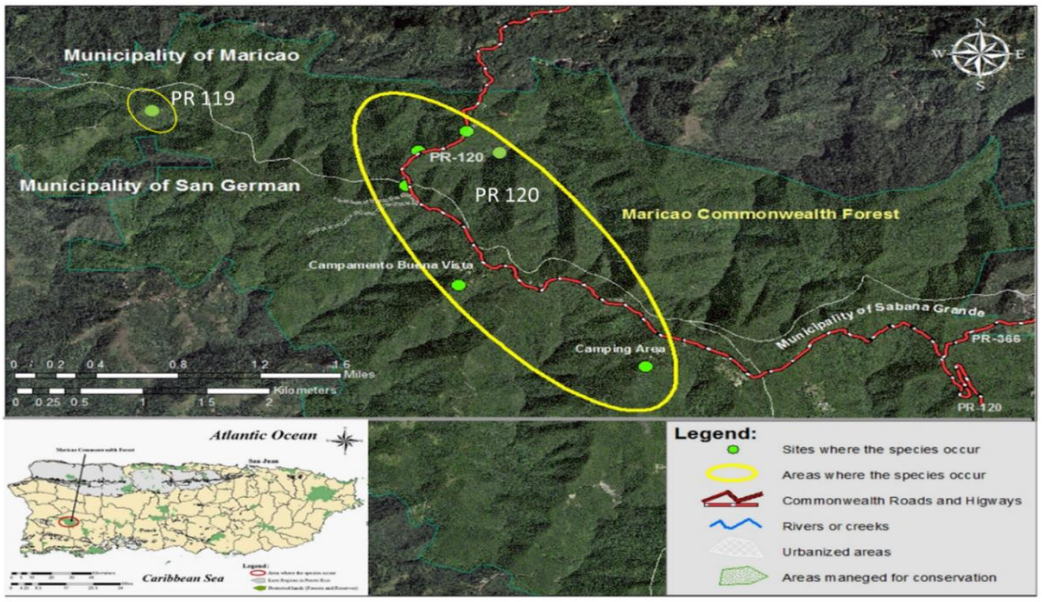


Figure 3-7. Map showing the distribution of the Puerto Rican harlequin butterfly at the Maricao Commonwealth Forest.

Susúa Commonwealth Forest Metapopulation

The Susúa Commonwealth Forest is situated between the municipalities of Sabana Grande and Yauco, approximately 9.5 km (5.8 mi) southeast of the Maricao Commonwealth Forest. Within the Susúa Commonwealth Forest, all life stages of the butterfly have been observed in two sections of the Eagle's trail, located in the southern section of the forest, as well as in one site situated in Cuchilla Larga sector in the northern section of the forest (Figure 3-8; Barber 2016, p. 12).

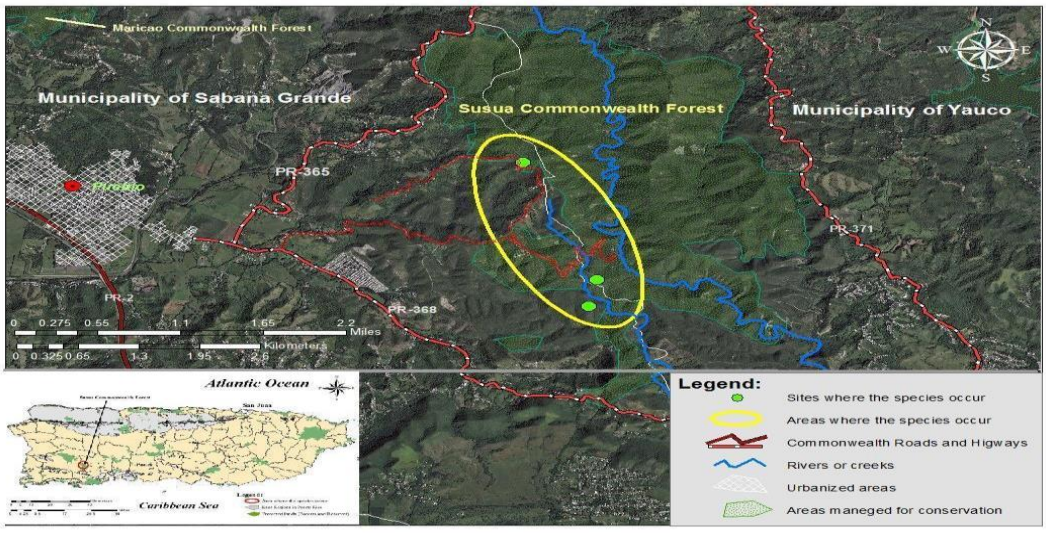


Figure 3-8. Map showing the distribution of the Puerto Rican harlequin butterfly at the Susúa Commonwealth Forest.

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

Region of Puerto Rico	Metapopulation	Number of subpopulations	Municipality	Amount of subpopulation per Municipality	Area of occurrence	Number of occurrences per site
Northern Karst Region	Isabela, Quebradillas, and Camuy (IQC)	13	Isabela, Quebradillas Camuy	6 6 1	Guayabo's Serctor Royal Isabela Los Milagros' Sector El Pastillo Cara del Indio El Tunel de Guajataca Tunel Negro El Merendero Terranova's cliff Puente Blanco San José' cliff Puerto Hermina Yeguadas' cliff	4 9 2 8 10 13 1 9 12 13 11 9 14
	Guajataca Forest	2	Isabela Quebradillas	1 1	La Caballas'Trail Private property at east of Guajataca River	6 4
	Río Encantado	3	Arecibo Florida Ciales	1 1 1	Sabana Hoyos Ward Alturas de Florida Frontón Ward	3 2 3
	Río Abajo Commonwealth Forest	1	Arecibo	1	PR 10 Campamento Radley	1 3
West-central Volcanic-Serpentine Region	Maricao Commonwealth Forest	2	Maricao San Germán	4 3	PR 120 PR 119	6 1
	Susúa Commonwealth Forest	2	Sabana Grande	2	Eagle's trail Cuchilla Larga Sector	2 1

Species Distribution Model

A species distribution models have become increasingly important tools for species conservation, particularly relevant for rare species whose habitat association are often not well understood (Ramírez-Reyes et al. 2022, p. 37). A species distribution model typically consists of a statistical model representing the environmental conditions associated with known species occurrences to estimate the geographic distribution of a species (Franklin 2013, p. 1217; Ramírez-Reyes et al. 2022, entire). Following this approach, Ramíres-Reyes et al. 2022 (entire) developed a species distribution models and weighted ensemble model for the Puerto Rican harlequin butterfly using

species occurrence data and a variety of predictor variable that can limit the distribution of the species. The ensemble model had great accuracy (Area Under the Curve (AUC)=0.92). Further, the ensemble model indicated 7.1% of the main island of Puerto Rico encompassed suitable habitat for the harlequin butterfly. However, only 0.5% was classified as including the greatest suitability. Using an ensemble modeling approach to delineate areas of the island with suitable environmental conditions may improve habitat conservation efforts for the species (Figure 3-9).

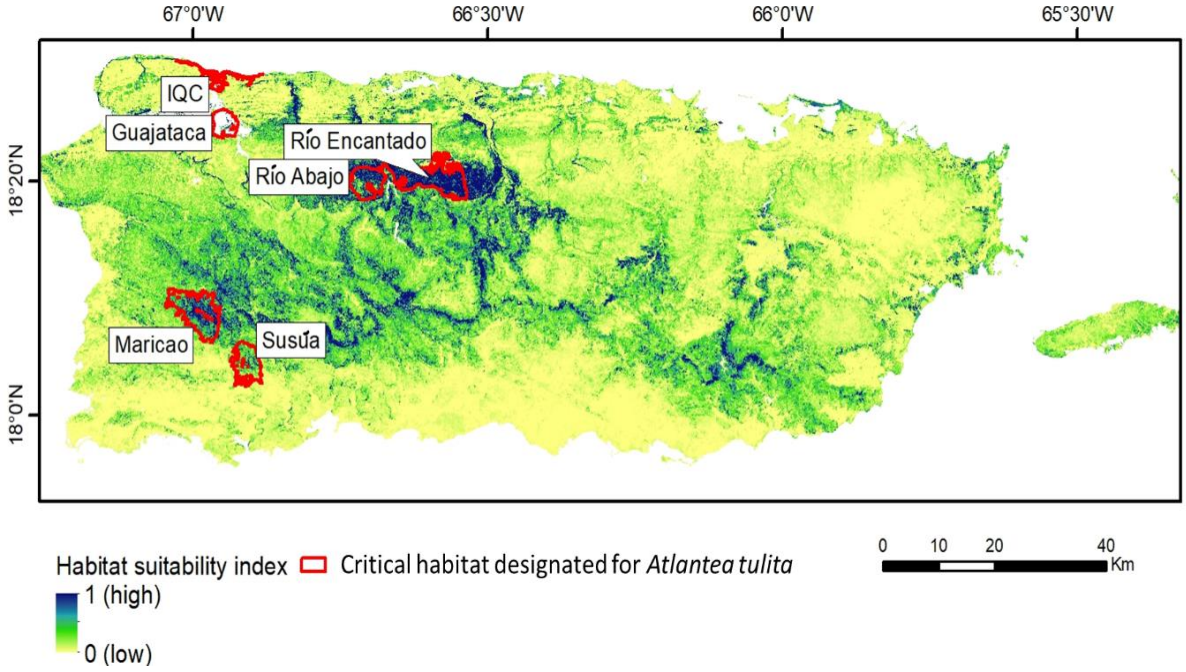


Figure 3-9. Map showing the locations where the Puerto Rican harlequin butterfly occupied habitat occurs in relations with the predicted areas with potential suitable habitat for the Puerto Rican harlequin butterfly in Puerto Rico (Ramírez-Reyes et al. 2022).

3.3 Abundance, Population Estimates

Currently, the information available on the abundance, population estimates, and trends of the Puerto Rican harlequin butterfly throughout its range is very limited or absent. Estimating the overall abundance, population densities, number of generations per year, or population trends for the Puerto Rican harlequin butterfly is challenging because existing biological studies have not been designed to determine these factors. As result, quantitative population size estimates (statically derived) for Puerto Rican harlequin butterfly, or for any of its metapopulations, are not available. The population estimates have been derived from anecdotal reports, expert opinions, and the number of individuals (imagoes and caterpillars) observed during single survey events. Consequently, the estimated abundance of the species per population may vary according to the methodology implemented during the survey and the sources of information used.

Currently, most data consist of counts of imagoes and caterpillars during single survey events. The most updated information on the species abundance suggests that in any given year the maximum number of Puerto Rican harlequin butterfly individuals recorded in its entire range is 200 butterflies (imagoes) and 2,096 caterpillars (Barber 2019, entire; Chabert 2023, p. 1). Although counts of imago and caterpillars are considered a reliable method to estimating butterfly abundance, we cannot assume that all individuals are counted, as not all life stages of the species are likely to be detected during a single survey. Furthermore, determining the size and structure of the Puerto Rican harlequin butterfly population is challenging due to the lack of information regarding factors that can affect the population growth (e.g., number of viable eggs per laying, mortality rates at each stage, among others). For example, the species may lay about 102 eggs per clutch, but subsequent counts after eclosion may yield a low number of imagoes (Service 2019, p. 32). Moreover, the presence of more than one generation observed during a survey confirms the species' multivoltine nature (producing several broods in a season) (Biaggi-Caraballo 2009, p.4). The lack of information on the population dynamics of the Puerto Rican harlequin butterfly is a limiting factor in defining what constitutes a viable population.

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

In IQC, The Puerto Rican harlequin butterfly occurs in 13 areas (subpopulations), but surveys for the species have been conducted in only 7 of those areas.

In the municipality of Isabela, surveys for the species have been conducted in 4 areas (subpopulations): 1. Royal Isabela, 2. El Pastillo, 3. Cara del Indio, and 4. El Guajataca Túnel. The number of Puerto Rican harlequin butterflies detected during a one-day survey in these areas has fluctuate from 0 to 200 imagoes (Chabert 2015, p.1; Barber 2019, entire; Service 2019, p.32;). From 2013 to 2023, the highest number of imagoes recorded at Isabela during a survey occurred in the months of March, April, May, October, and December, with observation ranging from 40 to 200 imagoes. Conversely, the lowest number of imagoes recorded was during the months of January, February, June, July, and November, with observations ranging from 0 to 18 imagoes (Chabert 2019, p.13, Barber 2019, entire, Service 2019, p 32; Chabert 2023, entire).

Royal Isabela is the area in the municipality of Isabela where the Puerto Rican harlequin butterfly imagoes are most often observed (Service 2019, p.32). J. Chabert, President of Fundación El Pastillo, often surveys an area of approximately 1.6 ha (3.9 ac) along the northern coastal cliff of Royal Isabela, reporting a total of 65 imagoes in 2013 and 50 imagoes in 2019 (Chabert 2019, p. 13, 14). More recently, in 2023, Chabert (2023, p.3) observed a total of 60 imagoes in March and 40 imagoes in April in the same area. The maximum number of imagoes ever recorded in a one-day survey in Royal Isabella has been over 200 imagoes in December 2014 in the same surveyed area (Chabert 2015, p.1; Service 2019, p.32). No other life stages of the Puerto Rican harlequin butterfly have been reported in Royal Isabela. In 2018, Barber (2019,

entire) surveyed for the Puerto Rican harlequin butterfly in other areas in Isabella (i.e., El Pastillo, Cara del Indio and El Túnel de Guajataca), recording a total of 13 imagoes and 153 caterpillars.

In the municipality of Quebradillas, the Puerto Rican harlequin butterfly is more often observed at El Merendero, Puente Blanco and Puerto Hermina. The number of Puerto Rican harlequin butterflies detected during a one-day survey in any of these sites may fluctuate from 0 to 50 imagoes (Service 2019, p. 32). In 2003, Carrión-Cabrera (2003, p.60) surveyed the species at Puente Blanco, observing 235 Puerto Rican harlequin butterfly imagoes over a 12-month period (with 2 sample days per month) on 0.34 ha (0.83 ac). In addition, Carrión-Cabrera (2003, p. 61) reported that larval counts from April to July and from December to January ranged between 100 and 200 caterpillars per survey day (involving 2 man-hours of search efforts) and were lower than 100 during the rest of the year (i.e., February and September to November) (Carrion-Cabrera 2003, p. 61). Later, in 2009, the number of Puerto Rican harlequin butterfly was estimated to be 45 or fewer imagoes on any given day in the same area surveyed by Carrion-Cabrera in Quebradillas (Biaggi-Caballero 2009, p.4). By 2011, the abundance of Puerto Rican harlequin butterfly in Quebradillas was estimated to be 50 imagoes and 100 caterpillars, or fewer, on any given day in the same area (76 FR 31282, May 31, 2011), resulting in a density estimate of 132 imagoes and 294 caterpillars per ha (or 54 imago and 120 caterpillars per ac) (Service 2019, p. 32).

Barber (2018 and 2019, entire) conducted a survey of the species following Hurricane María at six locations in IQC: El Pastillo, El Túnel de Guajataca, Cara del Indio, El Merendero, Puente Blanco and Puerto Hermina. The survey reported a maximum of 53 adults and 1,381 caterpillars across 2.68 ha (6.67 ac), yielding an estimated density of 20 imagoes and 515 caterpillars per ha (or 8 imagoes and 207 caterpillars per acre) (Table 3-2). The observation of multiple generations during the survey indicates that the species can produce several broods within a single season (Figure 3-10, below) (Biaggi-Caballero 2009, p. 4: Barber 2018, entire; Service 2019, p. 19). Since 2018, no further surveys of the Puerto Rican harlequin butterfly have been carried out in the mentioned sites. Nonetheless, the species, at all live stages, is frequently seen in these areas (Fourth Technical Meeting Puerto Rican Harlequin Butterfly Working Group, October 7, 2023; Pacheco 2023, personal observation). Moreover, the Puerto Rican harlequin butterfly is frequently observed along the Yeguadas cliff in Camuy. Nonetheless, no population estimate is available for this area, only sporadic sightings of the Puerto Rican harlequin butterfly (larva or imago). Therefore, the population size of Puerto Rican harlequin butterfly in the municipality of Camuy is uncertain.

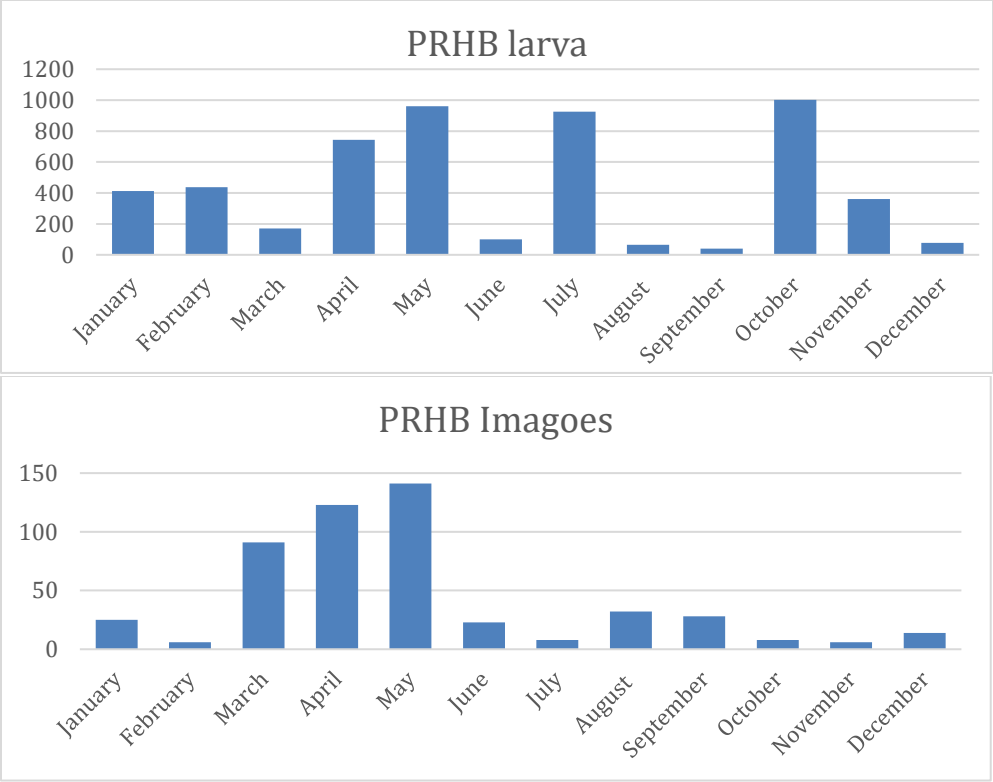


Figure 3-10. Maximum number of observed Puerto Rican harlequin butterfly caterpillars (top) and imagoes (bottom) per month throughout the year. Based on information provided by Carrion-Cabrera (2003), Monzon (2007), Biaggi-Caballero (2010), Barber (2018 and 2019) and Chabert (2023). This graphic excluded the maximum number of imagoes ever recorded in a one-day survey has been over 200 imagoes in December 2014.

The Puerto Rican harlequin butterfly has been poorly monitored in the Río Abajo Commonwealth Forest, Guajataca and Río Encantado. Therefore, the species abundance and the population size in these three metapopulations remain uncertain. The little available information on the number of Puerto Rican harlequin butterflies for these metapopulations is derived from sporadic sightings of the species (either larva or imago) rather than formal surveys.

In Río Abajo Commonwealth Forest, the Puerto Rican harlequin butterfly occurs in three sites (subpopulations), but surveys have only been conducted in one subpopulation located near Campamento Radley. In a survey conducted in June 2019, only 2 imagoes, 85 caterpillars, and 1 chrysalid of the Puerto Rican harlequin butterfly were observed in this subpopulation (Ríos 2019, entire; 87 FR 73655). Since then, no updated information on the species in Río Abajo Commonwealth Forest has become available.

The Guajataca metapopulation was discovered in July 2019, documenting 3 imagoes, 43 caterpillars and 3 chrysalides (87 FR 73655). In 2020, four new subpopulations were identified in Guajataca, observing all life stages of the Puerto Rican harlequin butterfly, but number of

imagoes and caterpillars were not provided (Monsón and Morales 2020, unpublished data, p. 1). Since then, the Guajataca metapopulation has not being monitored; thus, a population estimate is not available. However, frequent anecdotal reports of sightings of different life stages of the Puerto Rican harlequin butterfly in this metapopulation suggest the species is thriving in Guajataca area (Carlos A. Rodríguez, Liga Ecológica Quebradillana, 2023, personal communication, Fourth Technical Meeting Puerto Rican Harlequin Butterfly Working Group, October 7, 2023).

The limited information available for the Río Encantado metapopulation pertain only to sightings of different life stages (larva and imago) rather than the number of individuals observed (Service 2019, p. 33). Since 2019, no new information on the abundance, population estimates, or trends of the Puerto Rican harlequin butterfly in Río Encantado area have become available. Therefore, the abundance, population estimates, and trends of the Puerto Rican harlequin butterfly in Río Encantado area remain unknown.

Population estimates in the West-Central Volcanic-Serpentine Region: Maricao Commonwealth Forest and Susúa Commonwealth Forest

The Puerto Rican harlequin butterfly in the West-Central Volcanic-Serpentine Region has been poorly monitored. Therefore, information about population estimates and trends of the Puerto Rican harlequin butterfly in this region is limited or absent (Barber 2016, p. 15). Aside from the information provided below, no scientific data regarding the abundance of the species or populations trends are available.

In the Maricao Commonwealth Forest, the Puerto Rican harlequin butterfly is known to occur in seven sites (subpopulations), of which the species has been monitored at only two: Los Pinos and at La Cantera. These two sites are situated near the PR-120 and are less than 1km apart.

By 2011, the Puerto Rican harlequin butterfly population in the Maricao Commonwealth Forest was estimated to consist of no more than 20 imagoes and around100 caterpillars (76 FR 31282, May 31, 2011). After Hurricane Maria in 2017, Barber (2019, entire) conducted surveys over 12 months (March 2018-March 2019) at the two sites, Los Pinos and La Cantera, and found a maximum of 21 imagoes and a maximum of 632 caterpillars in 1.08 ha (2.67 ac), which equates to a density of 19 imagoes and 584 caterpillars per ha (or 8 imagoes and 236 caterpillars per ac). Since 2019, no updated information on the species' abundance in Maricao Commonwealth Forest has become available. Nonetheless, throughout the years, an undetermined number of imagoes and caterpillars of the Puerto Rican harlequin butterfly have often been observed in the Maricao Commonwealth Forest (C. Pacheco 2023, Service, pers. obs.).

The occurrence of the Puerto Rican harlequin butterfly in the Susúa Commonwealth Forest was first anecdotally reported in 2003 (Carrion 2003, p. 32). However, details regarding the location

of the sightings and the number of individuals observed were not provided (Service 2019, p. 33). Later in 2016, Barber (2016, pp. 12-15) documented one Puerto Rican harlequin butterfly imago and one larva in the Eagle's trail and in the Cuchilla Larga sector, respectively. More recently, Barber (2019, p. 56) surveyed these two sites documenting a maximum of 16 imagoes and 83 caterpillars over 1.08 hectares (2.67 acre) during the 12 months of surveys. This resulted in an estimated abundance of 15 imagoes and 77 larva per ha (or 6 imago and 31 caterpillars per ac) (Table 3-2). Since 2019, the Susúa Commonwealth Forest metapopulation has been poorly monitored. Nevertheless, recent anecdotal reports of sighting of different life stages of the Puerto Rican harlequin butterfly suggest that the species is persisting in the Susúa Commonwealth Forest (Pacheco 2023, personal observation).

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

Region of Puerto Rico	**Metapopulation**	**Maximum Number of Individuals Observed in one day survey**	**Surveyed Area**	**Source of Information**
Northern Karst Region	Isabela, Quebradillas and Camuy (IQC)	45 adults / 100 caterpillars	0.34 ha (0.83 ac)	Carrión-Cabrera 2003, p.34; Monzón-Carmona 2007, p.44; Biaggi-Caballero 2020, p.4.
		53 adults / 1,381 caterpillars	2.68 ha (6.67 ac)	Barber 2019, p.4
	Río Encantado	8 adults / 8 caterpillars	Not determined	Morales and Estremera 2018, unpublished data, p. 1; Service 2019, p. 28
	Río Abajo	2 adults / 85 caterpillars	Not determined	87 FR 73655
	Guajataca	3 adults / 43 caterpillars	Not determined	87 FR 73655; Monsón and Morales 2020, unpublished data, p. 1
West-Central Volcanic-Serpentine Region	Maricao Commonwealth Forest	21 adults or less / 0 to 631 caterpillars	1.08 ha (2.67 ac)	Barber 2019, p. 4
	Susúa Commonwealth Forest	16 adults or less / 0 to 83 caterpillars	1.08 ha (2.67 ac)	Barber 2019, p. 4

3.4. Habitat Description

Habitat descriptions at the subpopulation scale, including floral composition, the distribution of the prickly bush, and localities of the Puerto Rican harlequin butterfly eggs, caterpillars, or imagoes, are available for several sites and are provided in Appendix 1, 2 and 3 in the 2019 SSA ver 1.5 (Service 2019, p. 101-116).

Historically, the Puerto Rican harlequin butterfly has been found in three ecoregions: Northern Karst Region, Central-Western Volcanic-Serpentine Region, and Southern Karst Region. Within these ecoregions, the species persists in four life zones (or ecological settings): (1) subtropical moist forest on limestone-derived soil; (2) subtropical moist/wet forest on limestone-derived soil; (3) subtropical wet forest on serpentine-derived soil; and (4) subtropical dry/moist forest on serpentine-derived soil (Service 2019, p.25, 87 FR 73655). These life zones are distinguished by mean annual precipitation and mean annual temperature (Ewel and Whitmore 1973, p.25; Helmer et al. 2002, p.169). See Figure 3-11 and 3-12. Through these ecoregions and life zones, the Puerto Rican harlequin butterfly inhabits four types of forest: mature secondary moist limestone evergreen and semideciduous forest, young secondary moist limestone evergreen and semideciduous forest, mature secondary dry and moist serpentine semideciduous forest, and young secondary dry and moist serpentine semideciduous forest (Service 2019, p. 25; 87 FR 73655). These types of forest can be found 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 Puerto Rican harlequin butterfly reproduction and development.

Regardless of the life zone and forest type, the parches of native forest that the Puerto Rican harlequin butterfly occupies are characterized by canopy cover ranging from 50 to 85 percent, an average canopy height of 6 meters (m) (20 feet (ft)), and the host plant "prickly bush" covering more than 30 percent of the understory (87 FR 73657). In addition to prickly bush, several others native plant species that serve as sources of nectar for adult Puerto Rican harlequin butterflies are often found. A list of woody plant species known to occur in areas inhabited by the Puerto Rican harlequin butterfly is provided in appendix I. All the sites where the Puerto Rican harlequin butterfly occurs have a close (within a 1 km radius) water source (e.g., creek, river, pond, among others). Water and nectar sources for adult Puerto Rican harlequin butterfly may vary according to the life zone and habitat type. Furthermore, 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. Although the life history for the Puerto Rican harlequin butterfly and its ecological requirements for survival has not been well studied, it seems that these forest types provide suitable habitat conditions (e.g., shelter, moisture, temperature) for the species' persistence.

In some areas where the butterfly 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 Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly occurs are relicts of mature forest that might have survived the massive deforestation of 19th and early 20th centuries (Morales and Estremera 2018, p. 1; Barber 2019, p. 37).

The IQC metapopulation occurs in the subtropical moist forest life zone. Within this life zone, the species persists in forested habitat composed of young secondary lowland moist limestone evergreen and semideciduous forest and mature secondary lowland moist limestone evergreen and semideciduous forest (Gould et al. 2008, p. 14; Martinuzzi et al 2013, p. 84). The forest structure may vary according to their location through this area. At the cliff edges, the vegetation is constantly swept by trade winds from the ocean; as a result, the trees are stunted, most are sclerophyllous and the forest is very thick, making it most cases impenetrable. Moving from the cliff edge to the south, the canopy coverage increase from zero (0) percent up to 70 percent, and the mean tree height reaches around 6 m (20 ft) (Barber 2019, p.37; Vargas 2019, p. 2).

The metapopulations in Guajataca, Río Abajo and Río Encantado, all, occur is in the subtropical moist/wet-northern limestone forest life zone (Helmer et al. 2002, p. 169; Martinuzzi et al 2013, p. 84). The habitat in these metapopulations is composed of mature secondary moist limestone evergreen and semideciduous forest (Gould et al. 2008, p.14). The forest structure seems to be contiguous with little changes through the area. The habitat harboring the Puerto Rican harlequin butterfly metapopulations contain all the northern karst region forest habitat types and components of those habitat types that are the essential physical and biological features for the species.

The Maricao Commonwealth Forest metapopulation is in the subtropical wet forest life zone on serpentine-derived soil and contains three types of forest: (1) Mature secondary montane wet serpentine evergreen forest, (2) wet serpentine shrub and woodland forest, and (3) mature secondary montane wet non-calcareous evergreen forest (Helmer et al 2002, p. 169; Gould et al. 2008, p.14). The Susúa metapopulation is in the subtropical moist and subtropical wet forest life zones and contains mature secondary dry and moist serpentine semi-deciduous forest and young secondary moist serpentine evergreen and semi-deciduous forest (Gould et al, 2008, p.14; Martinuzzi et al 2013, p. 84).

For all these areas, special management considerations or protections may be required to address land conversion for rural developments, road construction and maintenance, utility and communications structures and corridors, and agriculture; fires and illegal garbage dumps (which are often the source of fires); and shifting environmental conditions and severe drought.

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 Puerto Rican harlequin butterfly (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 Puerto Rican harlequin butterfly (see Chapter 4- Factors Influencing the Species).

Presently, the fate of the Puerto Rican harlequin butterfly in the Southern Karst Region is unknown because the species has not been found since 1926 (87 FR 73655). Although the species has not been detected for many years in the subtropical dry forest in Southern Karst, this region should be considered potentially suitable habitat for the Puerto Rican harlequin butterfly because it harbors the prickly bush and some of the plant species that the butterfly life stages feed upon (Carrion 2003, p. 31).

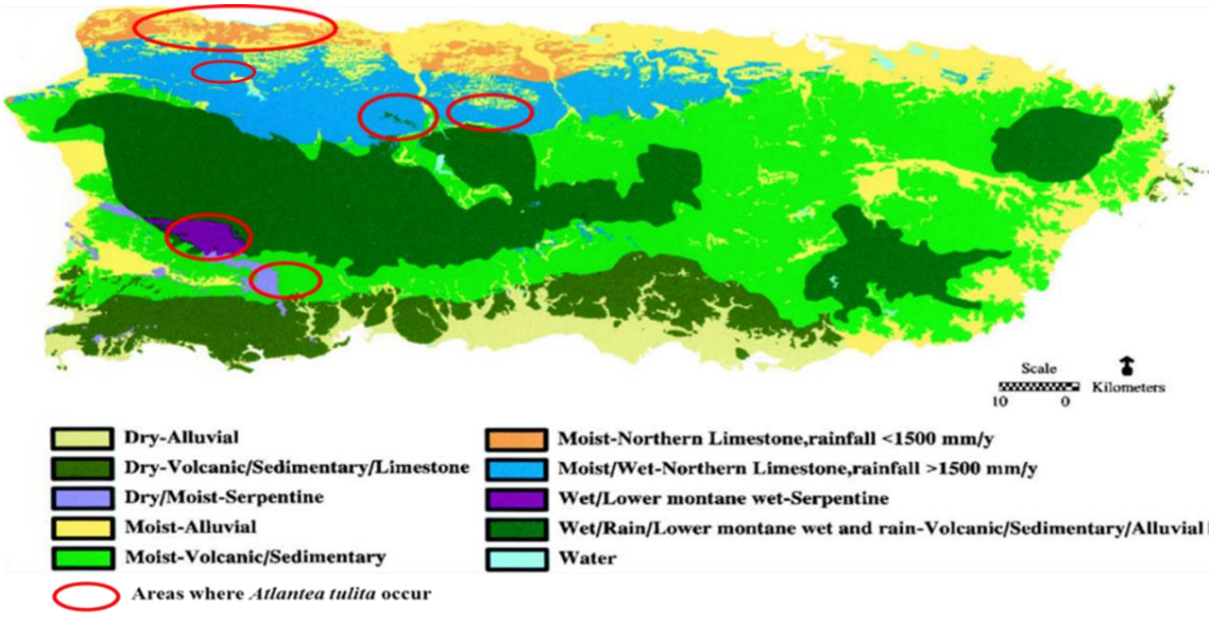
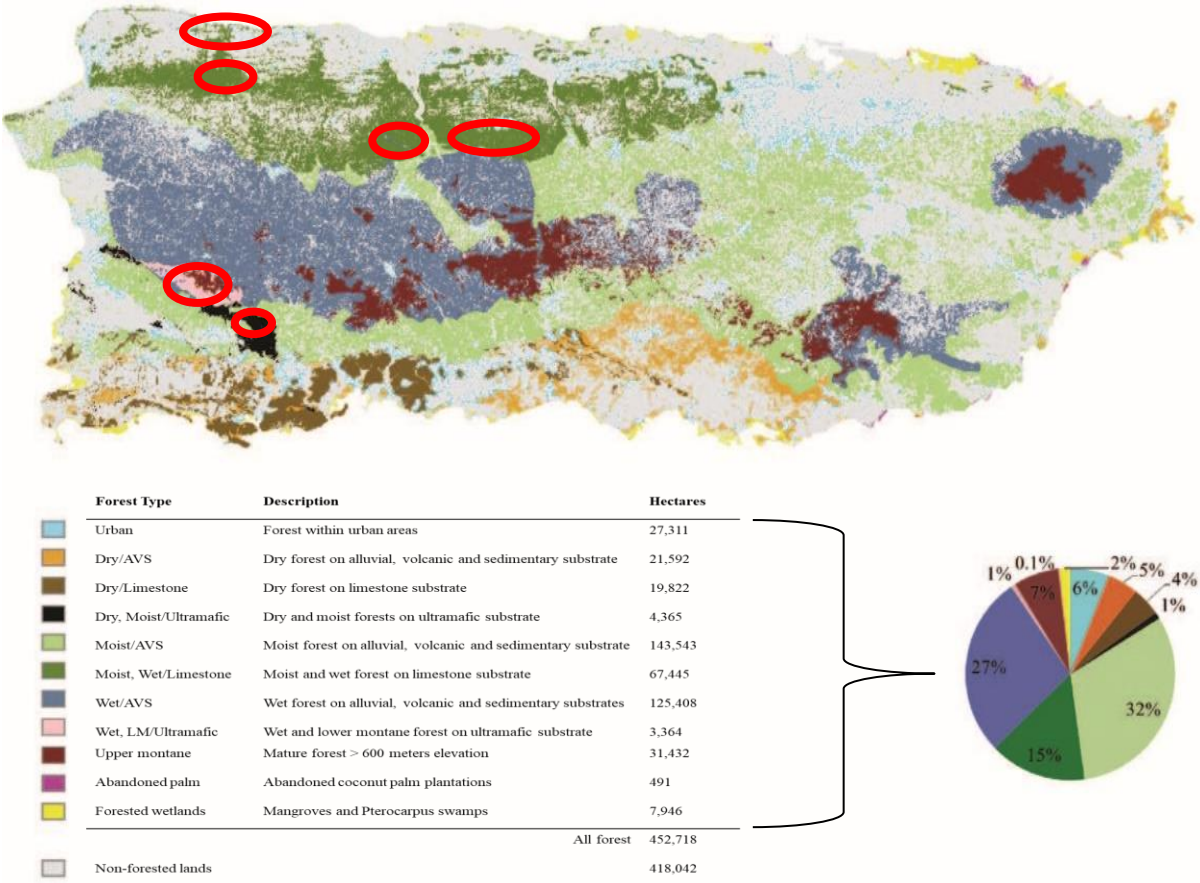


Figure 3-11. Map showing the locations where the Puerto Rican harlequin butterfly occurs in relation with the different forest type and life zones in Puerto Rico. (Helmer et al 2002, p. 169).



Forest Type	Description	Hectares
Urban	Forest within urban areas	27,311
Dry/AVS	Dry forest on alluvial, volcanic and sedimentary substrate	21,592
Dry/Limestone	Dry forest on limestone substrate	19,822
Dry, Moist/Ultramafic	Dry and moist forests on ultramafic substrate	4,365
Moist/AVS	Moist forest on alluvial, volcanic and sedimentary substrate	143,543
Moist, Wet/Limestone	Moist and wet forest on limestone substrate	67,445
Wet/AVS	Wet forest on alluvial, volcanic and sedimentary substrates	125,408
Wet, LM/Ultramafic	Wet and lower montane forest on ultramafic substrate	3,364
Upper montane	Mature forest > 600 meters elevation	31,432
Abandoned palm	Abandoned coconut palm plantations	491
Forested wetlands	Mangroves and Pterocarpus swamps	7,946
	All forest	452,718
Non-forested lands		418,042

Figure 3.12. Map showing the locations where the Puerto Rican harlequin butterfly occurs in relation with the different forest type in Puerto Rico. From Martinuzzi et al 2013 (p. 84).3.6

3.5 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 Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly population resiliency (Figure 3-13).

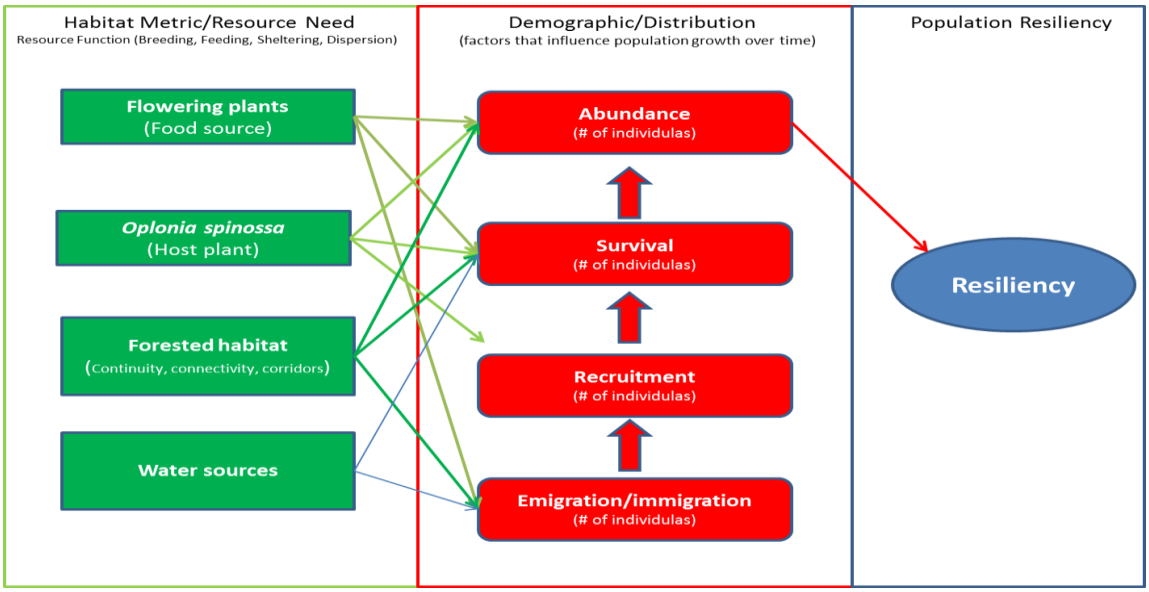


Figure 3-13. Basic conceptual model (or influence diagram) showing what resources may influence the resiliency of the Puerto Rican harlequin butterfly.

-Interaction or Connectivity Among the Populations.

As discussed above (section 3.3), the Puerto Rican harlequin butterfly is known from six (6) metapopulations: four (4) in the northern karst region and two (2) in the west-central volcanic region. It is likely that these metapopulations 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 Puerto Rican harlequin butterfly metapopulation function, but we are uncertain of the relative importance of each factor.

-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). Moreover, Carrion-Cabrera 2003 (p.46) also found that the number of the Puerto Rican harlequin caterpillars decreased as the number of imagoes increased, suggesting that the population dynamics of the species may be synchronized with a yet undetermined environmental factor. 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 Puerto Rican harlequin butterfly 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 (number of individuals counted on a determined time) as an indicator of population resiliency. Barber (2018, p. 1) reported densities of 20 imagoes and 515 caterpillars per hectare (or 8 imagoes and 207 caterpillars per acre) in Isabela, Quebradillas and Camuy; 19 imagoes and 584 caterpillars per hectare (or 8 imagoes and 236 caterpillars per acre) in the Maricao Commonwealth Forest; and 15 imagoes and 77 caterpillars per hectare (or 6 imagoes and 31 caterpillars 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 caterpillars per hectare (or 8 adults and 200 caterpillars per acre). However, the sex ratio of a natural Puerto Rican harlequin butterfly population is unknown.

-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 Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly over time is unknown. Monitoring conducted in three (3) of the six (6) populations suggests that there are inter-annual fluctuations in the Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly 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 prickly bush, amount and quality of adult food sources

and low presence of predators, plays an important role in Puerto Rican harlequin butterfly colonization success.

-Habitat Considerations

In addition to population size, the capacity for the Puerto Rican harlequin butterfly 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 habitat of the Puerto Rican harlequin butterfly 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). Throughout these habitat types, the species is found in forest patches with canopy cover ranging from 50 to 85 percent, and an average canopy height of 6 m (20 ft). In these forest patches, prickly bush coverage of more than 30 percent is an essential for the Puerto Rican harlequin butterfly. Connectivity among suitable forest patches and water sources is also essential for the species.

On December 1, 2022, the Service published the final rule designating critical habitat for the Puerto Rican harlequin butterfly (87 FR 73655), and identified the following primary constituent elements of the critical habitat for the species:

(A) *Forest habitat types in the Northern Karst region in Puerto Rico:* Mature secondary moist limestone evergreen and semi-deciduous forest, or young secondary moist limestone evergreen and semi-deciduous forest, or both forest types, in subtropical moist forest or subtropical wet forest life zones.

(B) *Forest habitat types in the West-central Volcanic-serpentine region in Puerto Rico:* Mature secondary dry and moist serpentine semi-deciduous forest, or young secondary dry and moist serpentine semi-deciduous forest, or both forest types, in subtropical moist forest or subtropical wet forest life zones.

(C) *Components of the forest habitat types.* The forest habitat types described in 1. and 2., above, contain: (i) Forest area greater than 0.4 ha (1 ac) that is within 1 km (0.6 mi) of a water source (stream, pond, puddle, etc.) and other forested area. (ii) Canopy cover between 50 to 85 percent and canopy height ranging from 4 to 8 m (13.1 to 26.2 ft). (iii) Prickly bush covering more than 30 percent of the understory.

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

3.6. 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 them 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 Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly 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, the greater the number of populations and the more widely distributed, the more redundancy the Puerto Rican harlequin butterfly 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 Puerto Rican harlequin over time are preserved.

Presently, the genetic diversity of the Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly, 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 Puerto Rican harlequin butterfly (Figure 4-1). 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 Puerto Rican harlequin butterfly 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. 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". 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).

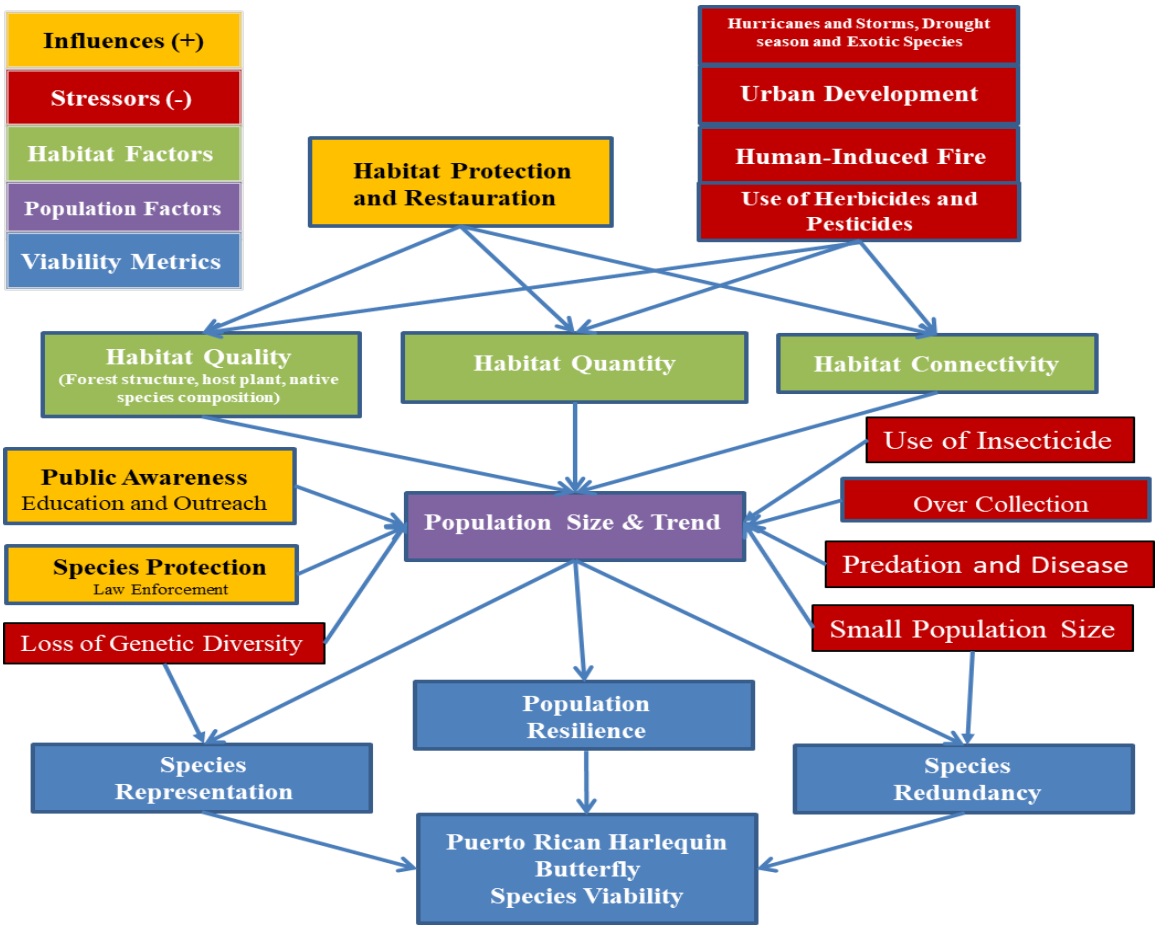


Figure 4-1. Key habitat factors, population factors, and supportive influences and stressors on viability used to assess resiliency, redundancy, and representation for the Puerto Rican harlequin butterfly.

Influences on the Puerto Rican harlequin butterfly vary from location to location, but stressors include habitat loss and modification by development, clearing vegetation as maintenance activity, predators, human induced fires, changing climate, pesticides and poor enforcement of existing regulatory mechanisms are wider spreader through its range. These stressors were analyzed in terms of their threats to the species' viability and were then considered as a reason for listing (87 FR 73655). Positive influences on the Puerto Rican harlequin butterfly have been habitat protection, habitat enhancement by reforestation and changes in habitat use, and public awareness.

4.1 Reason for Listing / Stressors Assessment

On December 1, 2023, the Service listed the Puerto Rican harlequin butterfly as threatened throughout its range due to threats of habitat loss and modification by urban and tourist developments, mechanical clearing of vegetation, use of pesticides (insecticides and herbicides), human-induced fires, small population size, changing climate, and insufficient enforcement of

existing regulatory mechanisms. During the listing process we used the term "threat" to refer in general to actions or conditions that are known to or are reasonably likely to negatively affect individuals of a species, defined above as stressors. A detailed evaluation of factors affecting the species can be found in the listing determination rule (87 FR 73655) and in the 2019 Species Status Assessment (Service 2019, p. 44). In this SSA report, we provide an updated threats assessment to the Puerto Rican harlequin butterfly as they related to the five listing factors outlined in section 4 (a)(1) of the Act.

- ***Present or threatened Destruction, modification and fragmentation or curtailment of the Puerto Rican harlequin butterfly habitat or range (Factor A).***

When the Puerto Rican harlequin butterfly was listed, the Service identified habitat modification, fragmentation and loses caused by urban and touristic development, agricultural practices, human induced fires, land management regimen (vegetation removal) and uses of pesticides (herbicides and insecticides) as factors influencing the decline of the Puerto Rican harlequin butterfly and its habitat (87 FR 73655). Presently, we believe these stressors continue to be an important factor threatening the survival and recovery of this species.

Relative to its historical range, the Puerto Rican harlequin butterfly's current range may reflect a remnant population of a once widely distributed forest dwelling butterfly whose habitat was decimated by historic Puerto Rican land uses. During European colonization, Puerto Rico was extensively modified for agricultural practices; as a result, by 1940, more than 90 percent of native forest in Puerto Rico were cleared for at one point in time (Miller and Lugo 2009, p.33; Pacarella et al 1999, p. 217; Terry et al. 2023, p. 1). The conversion of native forests into agricultural lands, and in recent history, conversion of these to urban and touristic developments has been the most significant modification and loss of suitable habitat for the Puerto Rican harlequin butterfly. The history of human occupation and land alteration is a prominent determination of current condition throughout the island (Brown and Lugo 1990, entire; Aide et al 1996, entire; Soto-Berelov and Edsall 2011, entire; Martinuzzi et al 2013, entire; McGinley et al. 2019, p. 1). Now, the species range has been reduced, and its habitat has been decimated resulting in a range that is now fragmented among discrete remnants of native forest.

The consequences of the loss and fragmentation of natural habitat for the Puerto Rican harlequin butterfly have been considered as detrimental because the species seems to have low dispersal capabilities, a limited "patchy" distribution, and specialized ecological requirements, including laying eggs and feeding only on prickly bush. Moreover, the species' rarity exacerbates the threats associated to this factor.

<u>-Agricultural, Urban and Tourist Developments</u>

Habitat modification, fragmentation and loss caused by agricultural, urban, and touristic development are considered the main factors influencing the decline of the Puerto Rican

harlequin butterfly (87 FR 73655). Habitat modification, fragmentation and loss in or around Puerto Rican harlequin butterfly populations would directly and indirectly fragment and impact its habitat and would limit its population expansion through the area (Figure 4-2 and 4-3).

The conversion of native forests into farms, pastures, or cropland, and in recent history, conversion of these to urban and touristic development has been the most significant change in suitable habitat for the species. As result, most of the suitable habitat for the species is currently fragmented by residential and tourist development and now the species faces significant threats from the existing and imminent destruction, modification, and curtailment of its habitat and geographic range (Service 2019, p.46). According to data from the 2010 Population and Housing Census, the human population density in the Northern Karst region increased 5.2 percent during the period from 1990 to 2010 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. (PRPB 2010, p. 45; PRAPEC, 2013, p. 18). Furthermore, available information indicate that housing has increased by 30.7 percent in the region in three decades: in 1980, there were 762,485 housing units, and in 2010, the number of units had increased to 1,101,041 (PRAPEC 2013, p. 19). Continued habitat loss and modification due to urban growth in both private and public lands in rural areas where the Puerto Rican harlequin butterfly resides are expected to occur over the next 25 years (Kennaway and Helmer 2007, p. 371; Soto-Berelov and Edsall 2011, p. 10; Castro-Prieto et al. 2017, p 478).

For instance, the IQC metapopulation occurs in suitable habitat located within an area classified by both municipalities of Isabela and Quebradillas, as well as by 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 with the potential for development to promote tourism due to its natural features and historic value. The IQC metapopulation occurs on both private and publicly owned lands, with only three (3) of the 13 subpopulations located in areas that may provide some level of protection due to being managed for conservation, passive recreation, or as scenic areas. For example, El Merendero, El Pastillo, and Royal Isabela are public and private lands where the managers or owners have recognized the presence of the species on their properties and have adopted measures to avoid negatively affecting the Puerto Rican harlequin butterfly (87 FR 73655, Chabert 2023, p.1). However, the other 10 subpopulations are on private land subject to be developed.

Available information indicates that housing along the municipalities of Isabela, Quebradillas and Camuy has increased by 54.1 percent over the last three decades: in 1980, there were 20,553 housing units; by 2010, that number had risen to 43,993; and in 2019, it increased to 44, 798 (PRAPEC 2013, p. 19; Puerto Rico Planning Board, jp.pr.gov/censo-2020, accessed on December 11, 2024). In 2013, over 20 residential and tourist development projects were proposed around the species’ occupied habitat, possibly affecting 29.4 ha (72.6 ac) in Quebradillas (Service 2019, p.46). From 2017 to the present, eight new houses, complete with

pools and gazebos, have been constructed, along with an additional gazebo on the edge of the cliff, and another house is currently under construction in the Puente Blanco area (Estremera, Líga Ecológica Quebradillana, 2023, personal communication). Three of these houses and their recreational facilities were built in areas where the Puerto Rican harlequin butterfly and the prickly bush were documented to be present by Barber (2018, p.2). This indicates that the species' habitat can be modified or lost due to land development for individual houses and small-scale residential and touristic projects occurring within and around the Puerto Rican harlequin butterfly habitat in IQC area (Service 2019, p.53). The ongoing conversion of the remaining forested habitat to urban and touristic development, roads, recreational parks, and golf courses will promote habitat fragmentation and loss, resulting in significant changes to the suitable habitat available for the species in the IQC area. This may represent a challenge for the Puerto Rican harlequin butterfly and could negatively impact the species and its recovery.

Figure 4-2. Photos showing signs of properties for sale and new projects along "Calle La Estación" in San José ward, Quebradillas. Photos showing some of the effects to Puerto Rican harlequin butterfly habitat caused by urban developments (Service 2019, p. 48).

The Puerto Rico harlequin butterfly also occurs in area 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 of these metapopulations 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.

Although the Río Abajo, Río Encantado and Guajataca, are covered and regulated 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 and its surroundings have experienced an increase of rural developments. According to Castro-Prieto (et al. 2017, p. 473), by 2016, a total of 32,300 new houses were constructed within 1 kilometer of protected areas, while the human population declined within the same area. The author suggests that the number of houses around protected areas will continue increasing while human population in Puerto Rico is declining both around protected areas and island wide. Many of these new houses or the development of rural communities require construction of additional

infrastructure (e.g., access roads, water supply service, among others) or improvement of the existing one, increasing their total effect to the surrounding habitats. Moreover, 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).

New construction and improvement of access road to existing or future rural communities may be considered as stressor that could directly (kill individuals and destruction of host plant) or indirectly (habitat degradation and fragmentation due to increase of traffic) reduce the populations of the Puerto Rican harlequin butterfly and its habitat. In fact, some of the Puerto Rican harlequin butterfly subpopulations in IQC, Guajataca, Río Encantado, Maricao and Susúa are located at less than 5 m (16.4 ft) from an access road. Please see figure 4-3. 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; Service 2019, 47; 87FR73655). Furthermore, removal of vegetation for construction of a new access roads and maintenance of the existing roads to private and public properties have been identified as a factor that could reduce the number of the Puerto Rican harlequin butterfly and its habitat in IQC, Maricao and Susúa (87 FR 73655). For example, the Puerto Rican harlequin butterfly and the prickly bush occurs adjacent (less than 5 m (15 ft)) of the edges of Calle La Estación and Calle Panoramica roads that provide access between El Merendero, Puente Blanco and Puerto Hermina in Quebradillas (Barber et al. 2018, p. 2). The expansion of the existing road will negatively affect the habitat and could result in loss of eggs, caterpillar and chrysalids of the Puerto Rican harlequin butterfly found in the host plant growing close and along to it (Service 2019, p.45) Additionally, clearing the native vegetation along the road may facilitate and accelerate colonization of exotic vegetation (i.e., invasive grass guinea grass (*Megathyrsus maximus*)) towards the butterfly habitat promoting fuel for wildfires (see Factor E discussion, below). Therefore, any improvement or expansion of this road would represent a threat to the Puerto Rican harlequin butterfly either by direct mortality or due to permanent loss, fragmentation, or alteration of its habitat.

Establishment of residential and tourist developments in rural regions will also lead to an increase in construction of or improvement of facilities for providing utilities such as power lines for transmission and distribution, water supply systems (e.g. water reservoirs and distribution pipelines), and communications infrastructure for cellular phone and related technologies including towers for cellular communication, internet connection, radio, television, and governmental purposes. As such, the installation for power transmission and distribution towers, telecommunication towers and water tanks pose a threat to plant species, including the prickly bush, that may occur on top of mogotes (limestone hills) or mountaintops where these facilities

are often situated. The proliferation of telecommunication towers has increased with the advent of cellular phone and related technologies. While these facilities 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. Moreover, the maintenance of these facilities, which includes clearing vegetation along security fences, access roads, and under power lines, may lead to habitat and host plant loss, and may result in direct mortality. Destruction or modification of this habitat may be irreversible, also causing permanent loss of the microhabitat conditions necessary for the recovery of the species.

Figure 4-3. Photo showing the location of host plants *Oplonia spinosa* occupied by the Puerto Rican harlequin butterfly (*Atlantea tulita*) with respect to the existing and improved roads. Photo A: Puerto Rican harlequin butterfly in road PR-4485 at Puente Blanco, Quebradilla; Photos B and C: Puerto Rican harlequin butterfly in road PR-120 in Maricao. Photos taken by Carlos Pacheco, USFWS, 2024.

-Land Management Regimen (Vegetation Clearance) and Use of Herbicides

Habitat fragmentation and loses caused by vegetation clearance is considered as another factor influencing the decline of the Puerto Rican harlequin butterfly and pose continuing threats to the species and its habitat (87 FR 73655). Removal of native vegetation for agricultural practices or changing the natural landscape in urbanized areas may result in both short- and long-term adverse effects to the Puerto Rican harlequin butterfly. Vegetation clearance within the occupied habitat by the Puerto Rican harlequin butterfly is one of the anthropogenic disturbance responsible in maintaining shifting matrix of suitable habitat and the essential features for the species. Habitat removal and alteration may have direct impacts and result in eggs, caterpillars and/or adults' mortality. The reproductive behavior of the Puerto Rican harlequin butterfly, including the cryptic nature of the species' caterpillars, 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 may be more suitable for the establishment of invasive exotic plant species that

may outcompete the prickly bush (host plant). Additionally, vegetation removal may result on changes in microhabitat conditions (e.g., promote more sunlight penetration that can change moisture conditions and temperature) that can affect the Puerto Rican harlequin butterfly in ways that are yet unknown given the lack of ecological information on the species. Furthermore, given the short dispersal capability of the species, the frequency and intensity of these disturbances shifting in habitat may promote local extirpations of the Puerto Rican harlequin butterfly subpopulations.

The Puerto Rican harlequin butterfly 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, especially promoted by loss of the prickly bush, feeding ground and shelter due to vegetation removal. Please see figure 4-4, below. Currently, the coastline of Isabela, Quebradillas and Camuy is under pressure for urban and tourist development, with only small remnants of coastal vegetation conserved in the steeper areas of the northern cliff still exist. Vegetation clearing along the roads and trails, in both private and public properties, and along the cliff to gain better views to the Atlantic Ocean may result in direct (e.g., killing some life stages of the species) and indirect (e.g., habitat loss) impacts to the species. For example, prickly bush grows on both sides of the existing hiking trails and around the picnic areas at El Merendero in Quebradillas. Maintenance personnel frequently trim the new growth of this plant to remove vegetation from the trails, picnic areas and from areas used by visitors as natural and scenic lookout, which may affect the harlequin butterfly, as it uses the newest vegetative branches of prickly bush for laying its eggs and feeding during the larval stages (Service 2019, p. 45) (Figure 4-5). Moreover, trimming the host plant and clearing the native vegetation in this area may result in mortality of the Puerto Rican harlequin butterfly eggs and caterpillars, and a reduction of breeding sites and food sources for adults. Currently, no guidelines regarding best practices for vegetation management and clearing have been developed to avoid or minimize effects to the species and its host plant in IQC.

In areas where undeveloped land remains, the species' larval food plant is likely to be affected by existing vegetation management practices that result in loss of suitable habitat for the species. Landowners and the municipality of Quebradillas have removed vegetation from their property and along roads, likely affecting the host plant, Puerto Rican harlequin butterfly individuals and the suitability of the recently designated critical habitat for the butterfly (Service 2019, p. 45; Isla Oeste 2023, entire).

Figure 4-4. Aerial photos of 2009 and 2023 showing changes in vegetation cover and land uses resulting in habitat loss of the Puerto Rican harlequin butterfly (*Atlantea tulita*) in Quebradillas (Service 2023, unpublished data).

Figure 4-5. Photo showing area where the vegetation clearance has negatively affected the Puerto Rican harlequin butterfly habitat and the host plants *Oplonia spinosa* in the Municipality of Quebradillas. Photo A, B, D and E: Vegetation cleared at both side of road PR-4485, Puente Blanco to Puerto Hermina, Quebradillas; Photos C: Puerto Rican harlequin butterfly habitat affected in El Merendero. Photos taken by Carlos Pacheco, USFWS.

-Use of Herbicides

Herbicides are used by conservation agencies, public agencies, and private organizations to control undesirable vegetation on right of way for roads, pipelines, drainage, and electrical transmission lines as a strategy to reduce the cost and frequency of maintenance (Atwood & Paisley-Jones 2017, entire; Mallick et al 2023, p. 346). Herbicides are also used by homeowners and managers of parks, golf courses and other lawns for aesthetic enhancement. Generally, herbicides are considered to have minimal effects on insects because the active ingredients target plants (Hoss et al. 2003, p. 110). However, the indirect effects of herbicides on the Puerto Rican harlequin butterfly are not well known.

Use of herbicides may result in both short- and long-term adverse effects to the Puerto Rican harlequin butterfly. The use of herbicides is a current threat to both the Puerto Rican harlequin butterfly and its host plant, which are found on the edges of roads and in the transition zone between open areas and forested habitat. The use of herbicides is a current threat to the Puerto Rican harlequin butterfly and its host plant in IQC, Maricao, Río Encantado, Guajataca and Susúa, where the host plant is found on the edges of roads, trails, under power transmission lines and on the edges of open areas. For example, 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 prickly bush (Figure 4-7; Service 2019, p.52, C. Pacheco, Service, 2024, personal observation). In Maricao, Río Encantado, Río Abajo, Guajataca and Susúa, the Puerto Rican harlequin butterfly and the prickly bush is found adjacent to and in the right of way of power transmission lines (Figure 4-7). If this practice is not carefully conducted, herbicides can indirectly impact Puerto Rican harlequin butterfly 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 (Mallick et al 2023, p. 349). In addition, the use of herbicides may promote the establishment in the area of weedy exotic species that may outcompete the prickly bush, thus, reducing the good quality of the habitat for the Puerto Rican harlequin butterfly.

The potential effects of herbicides on the Puerto Rican harlequin butterfly are strongly influenced by their toxic mode of action and their method of application. Direct applications may result in direct toxicity to non-target plants and animals or indirect effects due to alteration of the habitat on which the impaired species depends. The herbicides not only produce a lethal effect on herbs but also indirectly harm those species which use plants as host plants during their life cycle (Mallick et al. 2023, p. 350). Herbicides indirectly impair the growth and development of feeding caterpillars as well as the adult morphology, survivorship, and fecundity in Lepidoptera (Boggs and Freeman 2005, p. 359; Mallick et al. 2013, entire). For example, extensive use of glyphosate (Roundup) and glufosinate herbicides have been blamed to have impact on reducing the diversity of the butterfly population and for the decline in North America's monarch butterfly population by killing their host plant like milkweed (*Asclepias sp.*) (Arenas et al. 2020, p.2).

Moreover, the endangered Fender's blue (*Icaricia icarioides fender*), the threatened Oregon silver spot (*Speyeria zerene hippolyta*) and the Taylor's checkerspot (*Euphydrya sedithataylori*) are at high risk of decline due to the extensively use of herbicides through their habitat influencing the survival and larval development (Mallick et al. 2023, p. 346). Impairments to the butterfly are also more likely when herbicides are applied together or with other pesticides (Streibig et al. 1985, entire; Paveley et al 2003, p. 638), resulting in additive or synergistic effects. For example, Paraquat and Atrazine are both the most powerful and effective herbicides (weed killers) used in the United States. Both herbicides may react synergistically with insecticides such as Dursban, Brodan, Piridane and Eradex which has been widely used in home and on farms, resulting in this mixture in a product more toxic to invertebrates than the individual pesticides (Lydy and Linck 2003, p. 343). Atrazine also increased the effects of other pesticides on mosquito caterpillars (Belden and Lydy 2000, Lydy and Linck 2003, p. 349).

Habitat removal and degradation by use of herbicides and loss of individuals by use of insecticides may 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 species that may outcompete the Puerto Rican harlequin butterfly and the prickly bush.

Figure 4-7. Photos showing evidence of vegetation clearances using herbicide within the Puerto Rican harlequin butterfly habitat. Photos taken by Carlos Pacheco, USFWS.

Overall, the rarity and restricted distribution of the Puerto Rican harlequin butterfly, makes it vulnerable to habitat destruction and modification. The scope of these factors is exacerbated because the most significant portion of the known populations, (i.e., IQC population), occurs adjacent to urban developments, in a recreational facility and at the edge of the existing access road and trails. The maintenance activities (e.g., vegetation removal, use of pesticides in private and public properties and along the road) are expected to continue. Therefore, the Service believes that the Puerto Rican harlequin butterfly is currently threatened by this factor.

-Human Induced Fire

The Puerto Rican harlequin butterfly is exposed to fire incidences and to impacts associated with changes to the habitat on which this species depends (i.e., changes in microclimate conditions and food sources). At present, we have no information about the adaptive abilities of the species in such a situation. Therefore, the effect of wildfires within the Puerto Rican harlequin butterfly habitat should be considered a threat to the species.

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 Puerto Rican harlequin butterfly habitat and to the 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 Puerto Rican harlequin butterfly either temporarily or permanently and promote habitat fragmentation (Service 2019, p. 50). A fire may also have a direct impact on the Puerto Rican harlequin butterfly by killing imagoes, eggs, caterpillars, 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 Puerto Rican harlequin butterfly and its habitat in Quebradillas, Maricao and Susúa Commonwealth Forest (Service 2019, p.50). Wildfires resulting from natural or anthropogenic origin are growing and frequency across Puerto Rico (Brandeis and Woodall 2008, p. 558). Although wildfires may occur year-round, most wildfires on the island of Puerto Rico occur primarily in the first three months of the year, corresponding to the dry season. (Figures 4-8 and 4-9; 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 Puerto Rican harlequin butterfly.

The Puerto Rican harlequin butterfly occurs on the driest section of this forest, near road PR-120 and PR-119 in Maricao. On February 25, 2005, a human-induced burned more than 400 acres with unknown effects on the Puerto Rican harlequin butterfly population (Biaggi-Caballero 2010, p. 10, Service 2019, p.50). More recently, on March 9, 2023, a human-induced fire burned approximately 35 acres of the Puerto Rican harlequin butterfly habitat in Maricao (Figure 30, Ramos-Güivas 2023, entire). Both fires likely had at least temporary effects on the butterfly's habitat, but we have no information regarding these effects and whether 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 (Service 2019, p.50).

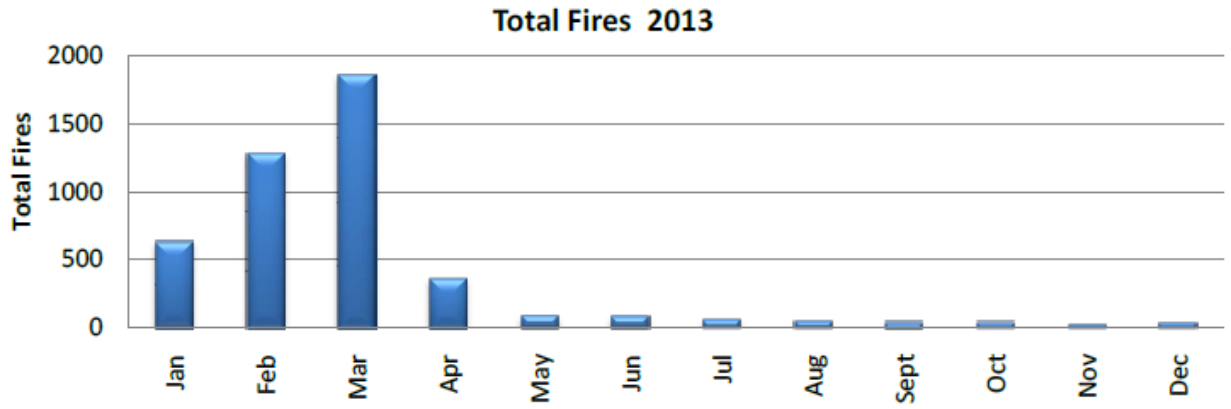


Figure 4-6. Wildfires per month that occurred in Puerto Rico during 2013. (Mendez-Tejeda et al 2015, p. 362).

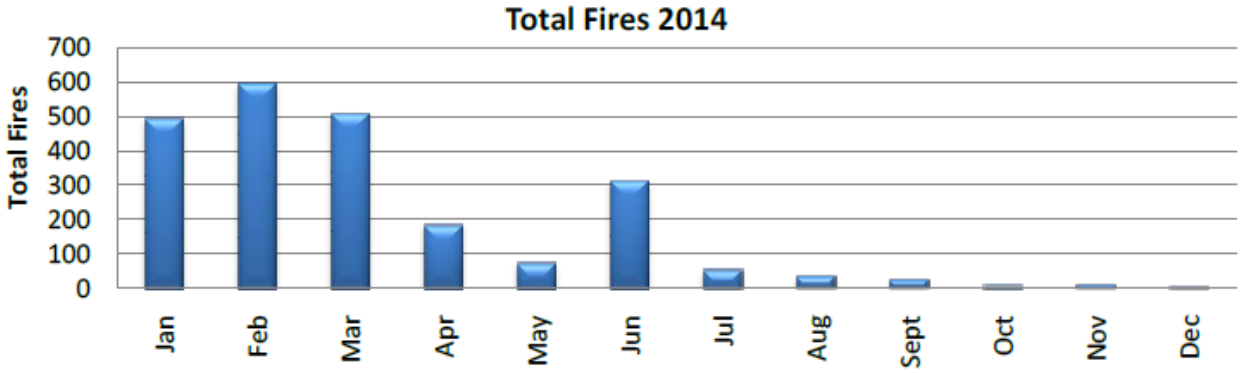


Figure 4-8. Wildfires per month that occurred in Puerto Rico during 2014. (Mendez-Tejeda et al 2015, p. 362).


Figure 4-9. Photos showing areas burned by human-induced fires in Quebradillas and Maricao Commonwealth Forest.

- ***Overutilization for Commercial, Recreational, Scientific, or Educational Purposes (Factor B)***

At the time of listing the Service determined that the Puerto Rican harlequin butterfly is not threatened by this factor because collection of the species is regulated by the Commonwealth Law No. 241 and Regulation 6766 (PRDNER 2004; PRDNER 2010). See Factor D, below. However, we evaluated the possible effects on the Puerto Rican harlequin butterfly of overutilization of the species for commercial, recreational, scientific, or educational purposes.

The Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly populations in many locations. Because there is significant uncertainty regarding the Puerto Rican harlequin butterfly biology (i.e., abundance, distribution, habitat requirements, genetics, and life history), any collection of imagoes, caterpillars, 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.

Prior to the listing, an undetermined number of Puerto Rican harlequin butterfly have been collected for scientific purposes and deposited in universities and private collections (Service 2019, p. 58). However, at present few researchers are working with the species, and its collection is regulated by the Puerto Rico Department of Natural and Environmental Resources. 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. Therefore, the Service continues to believe that the Puerto Rican harlequin butterfly is not currently threatened by this factor.

- ***Disease or Predation (Factor C)***

Disease or predation are not considered factors influencing the viability of the Puerto Rican harlequin butterfly. Presently, no diseases or predation has been documented to be affecting the viability of the species in the wild. However, due to the low number of individuals and known populations, disease and predation could certainly be a threat to the species. 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 Puerto Rican harlequin butterfly (Figure 4-10). 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 caterpillars 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 Puerto Rican harlequin butterfly (Carrión-Cabrera 2003, p. 41).

Although the Puerto Rican harlequin butterfly 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 species.

Figure 4-10. Potential predators of the Puerto Rican harlequin butterfly.

- ***Inadequacy of Existing Regulatory Mechanisms (Factor D).***

Inadequacy of existing regulatory mechanisms (Factor D) was considered as a stressor that can affect the viability of the Puerto Rican harlequin butterfly (87 FR 73655). Since listed, regulatory protection has become as another supportive factor for the conservation and recovery of the Puerto Rican harlequin butterfly.

The Puerto Rico Department of Natural and Environmental Resources (PRDNER) designated the Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly, 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.

The forested habitat that the Puerto Rican harlequin butterfly depend on in Río Abajo, Maricao and Susú Commonwealth Forests 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. The PRDNER also identified these Commonwealth Forests 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).

Although statutes discussed above provide legal protections for the Puerto Rican harlequin butterfly throughout its range, sometimes the enforcement of such legal mechanisms is challenging (see discussion under Factor A, above). For example, habitat loss (e.g., by cutting or pruning, or removing the host plants) or even extirpation of individuals of the Puerto Rican harlequin butterfly has occurred due to lack of knowledge of the species by staff from the municipality of Quebradillas during vegetation management in El Merendero and vegetation clearance along the access road to Puente Blanco Sector in Quebradillas. Similar situations occur when vegetation removal occurs during maintenance of the right of way of the power transmission lines facilities in the IQC, Guajataca, Río Encantado, Maricao and Susúa Commonwealth Forests.

- ***Other Natural or Manmade Factors Affecting its Continued Existence (Factor E).***

Other natural or manmade factors that may affect the continued existence of the Puerto Rican harlequin butterfly are analyzed as a variety of direct and indirect impacts on the species, impacts that can be exacerbated by the effects of other factors previously discussed in this review (See Factor A). In the Caribbean, endemic butterfly species, particularly rare species with limited distribution and highly specialized ecological requirements, may be vulnerable to stochastics events such as hurricanes, wildfires, vegetation management, the use of pesticides and changes

on environmental conditions. Therefore, these stressors could affect the continued existence of the Puerto Rican harlequin butterfly (87 FR 73655).

-Low Number of Individuals and Genetic Variation

The Puerto Rican harlequin is currently characterized by perennially low numbers of individuals (less than 100 imagoes observed per year). The apparently low reproductive rate (average lifetime number of offspring produced by a member of a population) of the Puerto Rican harlequin butterfly, its scattered and disconnected distribution 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 metapopulations.

Given the extremely low known number of individuals of the Puerto Rican harlequin butterfly, it is highly likely that its genetic variability is very low. 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 Puerto Rican harlequin butterfly to overcome stochastic events is available. However, it is well known that gene flow influences genetic health 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. deforested habitat for agricultural practices and urban areas) between them. Therefore, we consider the possible lack of genetic variation as a stressor to the species.

In the absence of knowledge on the natural recruitment capacity, survivorship at all life stages, and habitat fragmentation and genetic variability of this species, it is difficult to predict the recovery of the species after stochastics events such as hurricanes, wildfire, severe drought, and use of pesticides. Effects of stochastic events can be exacerbated by the low number of individuals known through entire range of the Puerto Rican harlequin butterfly. Thus, it is possible that any of the Puerto Rican harlequin butterfly metapopulation can be easily extirpated by a stochastic event.

-Use of Pesticides

Pesticides, which include herbicides, insecticides, and fungicides, are commonly used throughout the range of the Puerto Rican harlequin butterfly on crop fields, along public roads, and on private properties to control plant and animal pests. While pesticides are considered essential for pest control, their excessive use and application beyond the recommended dosage lead to severe consequences, becoming a serious health hazard for humans and causing negative impacts on the environment and wildlife (Garud et al 2024, p. 1) Presently, most Puerto Rican farmlands are

dominated by industrial agriculture, a system largely influenced by chemically intensive food production that depend on pesticides and chemical fertilizers (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 (Garud et al 2024, entire). Currently, the use of pesticides is recognized as adversely affecting the butterfly biodiversity and is one of the factors responsible for the declining butterfly abundance in the world (Sonoda et al. 2011, p. 335; Mallick et al. 2023, p. 346).

Puerto Rico has a long history of using pesticides, mostly insecticides for mosquito control in and around urban areas. Pesticides used to control invertebrates that pose a threat to human health or agricultural products may negatively affect the Puerto Rican harlequin butterfly. Pesticide impacts to the Puerto Rican harlequin butterfly are primarily influenced by the extent of the butterfly's exposure to pesticides throughout its range. Pesticides can be lethal killing eggs, caterpillars, and adults of the Puerto Rican harlequin butterfly during application of the chemical in the areas where the species occurs and can be sublethal to the butterfly when it is directly applied to vegetation on which the species depends for its development (prickly bush) and for food sources. Currently, no pesticide uses guidelines have been developed for application in areas where the Puerto Rican harlequin butterfly occurs (Service 2019, p. 50). Nevertheless, exposure to pesticides is expected to continue because insecticides and herbicides are the most effective tool for eradicating mosquitoes and control the overgrowth of some grass and woody vegetation.

Pesticides may adversely affect all life stages of the Puerto Rican harlequin butterfly across its geographic range, where is likely more prevalent in urban areas in IQC. In IQC, the Puerto Rican harlequin butterfly and the prickly bush is found in areas surrounded by residences. Use of pesticides may have direct impacts and result in eggs, caterpillars and/or adults' mortality. Fumigation programs are implemented by the local government authorities in the municipalities of Isabela, Quebradillas and Camuy to control mosquito-borne diseases (Biaggi-Caballero and López 2010, p.9). Thus, the fogging application of insecticides targeting mosquitoes (*Aedes aegypti* or *Aedes albopictus*) to control mosquito-borne diseases (e.g., Dengue, chikungunya and Zika virus) poses a risk of incidental harm to the Puerto Rican harlequin butterfly (Service 2019, p.50).

For example, in 1987, the Center for Disease Control (CDC) and the Puerto Rico's government carried out aerial spraying of naled across 177,000 acres of San Juan metropolitan area in attempts to control Dengue. Naled is an insecticide that has been registered since 1959 for use in the United States primarily for controlling adult mosquitoes, but also it is used for controlling other pest in greenhouses and crops (https://www.epa.gov/mosquitocontrol/naled-mosquito-control). For mosquito control, naled is most applied aerially as an ultra-low volume (ULV) spray. Regarding its risks to wildlife from aerial application of naled for mosquito control, it is

considered as minimal risk because naled is applied from several hundred feet above the ground, at low rates, and it does not persist in the environment. However, because naled is an insecticide, invertebrates such as butterflies, crustaceans, and spiders could be affected. In addition, wildlife present in the immediate treatment area could be affected shortly after spraying occurs but long-term effects are not expected. In February and March 2016, the CDC and Puerto Rico's government carried out again aerial spraying of naled but this time was over 14 different urban areas along the Puerto Rico island as a strategy to control the mosquito populations to control Dengue, chikungunya and Zika. The Environmental Protection Agency (EPA) is currently re-evaluating naled as part of its routine re-evaluation process, under which existing registered pesticides must by law be re-evaluated at least every 15 years to ensure they can be used safely, without unreasonable risks to human health and the environment. Currently, we have no information about the risk of aerial application of naled on the Puerto Rican harlequin butterfly, but we believe it could pose a negative effect on the species.

-Atmospherics Disturbances and Changes in Environmental Conditions

Atmospherics disturbances (e.g., hurricanes and storms) and changes in environmental conditions (e.g. temperature, humidity, and precipitation) can have a variety of direct and indirect impacts on Puerto Rican harlequin butterfly and its host plant and can exacerbate the effects of other stressors. Each one of the mentioned stressors may influence the viability of the species when considered in term of scale, intensity, and duration, as well as the impact of each one has on the species and habitat across the different life stages. Thus, rather than assessing "atmospheric disturbances" 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 these disturbances and various aspects of the Puerto Rican harlequin biology. Some stressors may affect the species at all life stages or all individuals across the species' range, while others may affect a specific life stage. Additional stressors acting on individuals of the species may not rise to the level of affecting the species or population(s).

Hurricanes or tropical cyclones are atmospheric disturbances that frequently affect the Caribbean Region. In fact, Puerto Rico is frequently in the path of hurricanes (Figure 4-11, Service 2019, p.58). Thus, as a species endemic to Puerto Rico, the Puerto Rican harlequin butterfly should be adapted to hurricanes and tropical storms, but its low number of individuals and small number of populations, place it at an increased risk, especially as it is predicted to increase the frequency and strength of tropical storms (Service 2019, p. 58).

In the last decade, Puerto Rico has been struck by three major hurricanes ranging from category 1 to 4. On September 6, 2017, Hurricane Irma passed approximately 100 km (62.1 mi) off the northeast coast of Puerto Rico bringing over 30 cm (11.8 in) of rain to some parts of the island (Hall et al 2020, p. 2). Nevertheless, no damage to the Puerto Rican harlequin habitat in Puerto Rico was reported. Two weeks later, on September 20, 2017, Hurricane María made landfall

along the southeast coast of Puerto Rico with maximum sustained winds of 210 km (130 mi) per hour, with up to 150 cm (59 in) of storm-related rainfall in some areas over a period of 48 hours, causing widespread damage to the island's forests (Hu et al. 2018, p. 827; Hall et al. 2020, p.2). On September 18, 2022, Hurricane Fiona hit the Puerto Rico area with maximum winds near 148 km (92 mi) per hour, with up to 40.6 cm (16 in) of storm-related rainfall in some areas over a period of 24 hours (Pasch et al. 2022, p.60). Recent studies suggest that forests growing at high elevations or on windward slopes were more exposed to high wind speeds and experienced greater damage and tree mortality from these severe storms (Uriarte et al. 2019, p.2, Hall et al. 2020, p.1). In fact, the forested habitat at the six localities where the species occurs suffered severe damage during the pass of Hurricane María and Hurricane Fiona, observing a massive tree defoliation and loss of branches (Barber 2018, Pacheco 2023, pers. obs.). However, although an undetermined number of individuals of the Puerto Rican harlequin butterfly could have been negatively affected by the impacts associated with Hurricane María and Hurricane Fiona, overall, the species seems to have overcome this impact and persists, and the habitat seems to be recovering well (87 FR 73655, Pacheco 2023, personal observation), although canopy height and density appear to be significantly reduced.

Hurricanes contribute to shaping vegetation and ecosystem processes, being it a factor in determining the structure and composition of biotic communities in the Caribbean Forest (Brokaw and Walker 1991, p. 442; Walker et al. 1991, p. 313; Lugo 2008, p. 368). Hurricanes can produce sudden and massive tree mortality, which is variable among species (Lugo 2000, p.245; Uriarte et al. 2019, p. 1), reducing food sources for the species. Hurricane winds often lead to tree defoliation, loss of small and large branches, and up-rooting, resulting in damage to adjacent trees and understory plants, like the prickly bush (Hall et al. 2020, p.1). Also, the loss of canopy allows direct penetration of sunlight, increasing temperature and altering the moisture conditions (Flynn et al 2010, p. 149), condition probably necessary for the egg and caterpillars' stages survival. Hurricanes can promote salinization of coastal soil from storm surge and associated changes in soil chemistry and change in soil nutrient pools and fertility (Lodge and McDowell 1991, p. 373). Therefore, an increase in intensity and frequency of hurricanes and tropical storms can modify the microclimate, the plant species composition of the Puerto Rican harlequin butterfly habitat, as well may affect the phenology of the prickly bush.

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 prickly bush habitat, promoting conditions for fires, and altering the microclimate and nutrient cycling of the habitat that is currently suitable for the Puerto Rican harlequin butterfly.

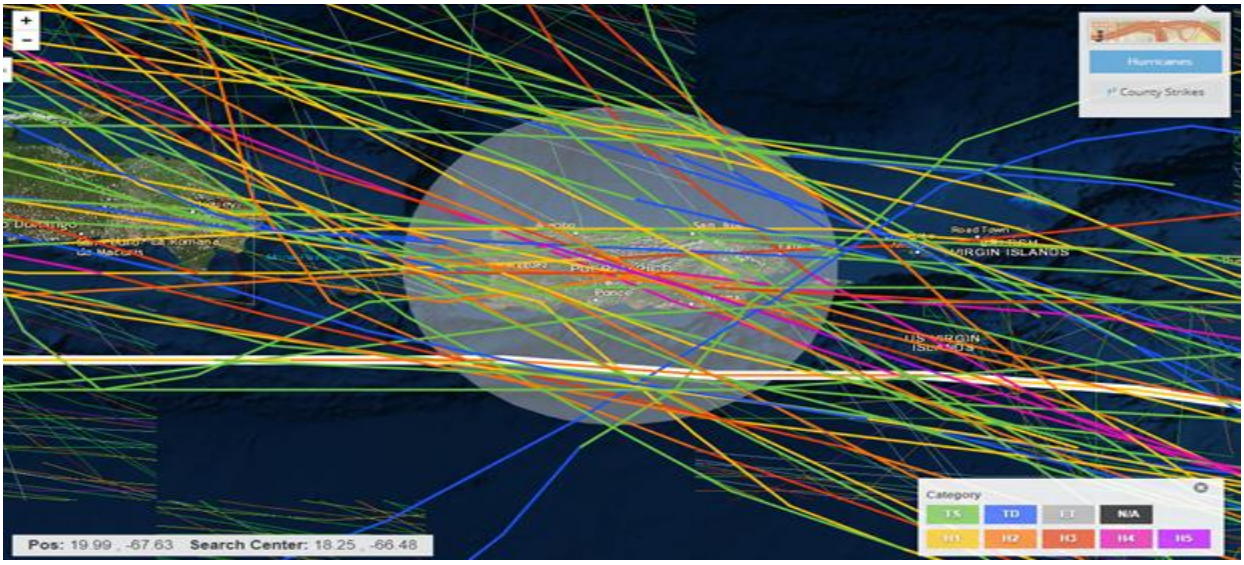


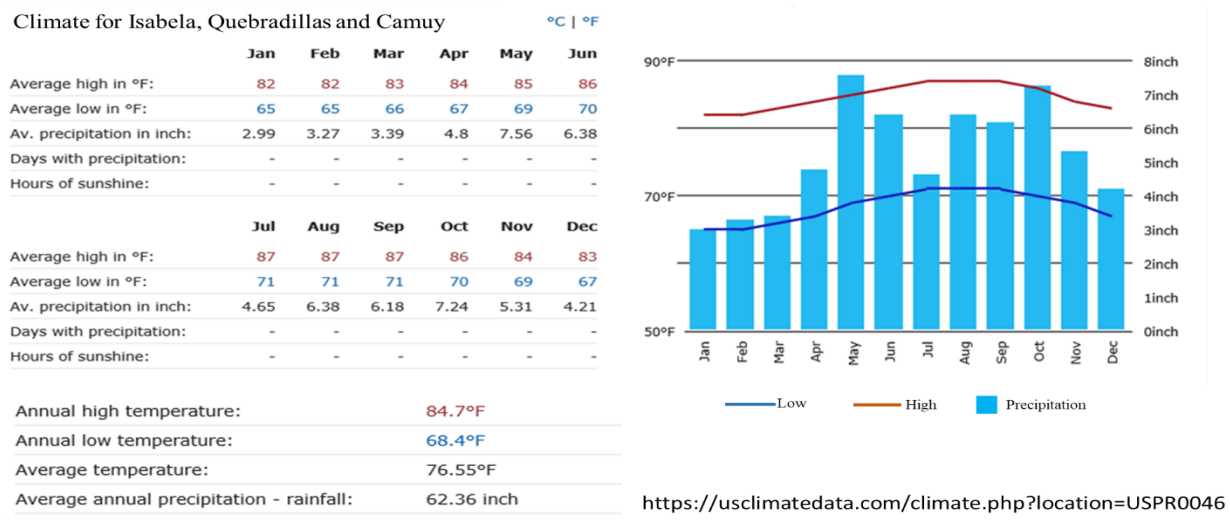
Figure 4-11. Historical hurricane tracks (late 1800s to current) in relation to Puerto Rico (NOAA 2018).

Vulnerability to environmental change impacts is a function of sensitivity to those changes, exposure to those changes, and adaptive capacity (USGCRP 2018, 20:821, Service 2019, p.54). 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 environmental change. As previously mentioned, the Puerto Rican harlequin butterfly 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 and precipitation), making the species susceptible to the effects of environmental 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 Puerto Rican harlequin butterfly (Figure 4-12, 4-13, 4-14, and 4-15) 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* caterpillars (Mathavan and Pandian 1975, p. 63). At 37ºC (98.6ºF) these same caterpillars 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* caterpillars, 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 Puerto Rican harlequin butterfly 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:	-	-	-	-	-	-

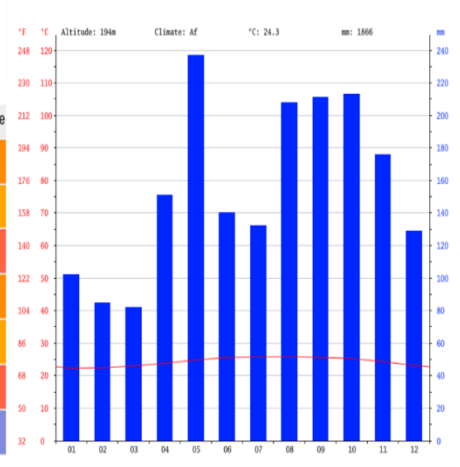
Annual high temperature:	84.7°F
Annual low temperature:	68.4°F
Average temperature:	76.55°F
Average annual precipitation - rainfall:	62.36 inch

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

Figure 4-12. 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/

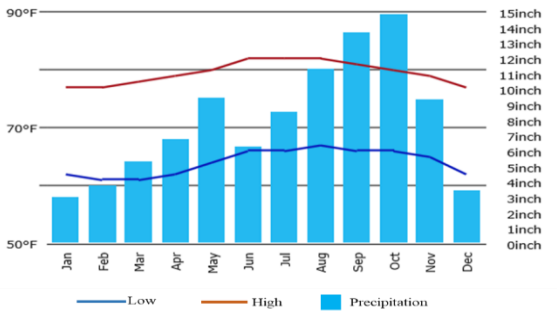
Figure 4-13. Climate data for the Rio Abajo Commonwealth Forest and Rio Encantado

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:	-

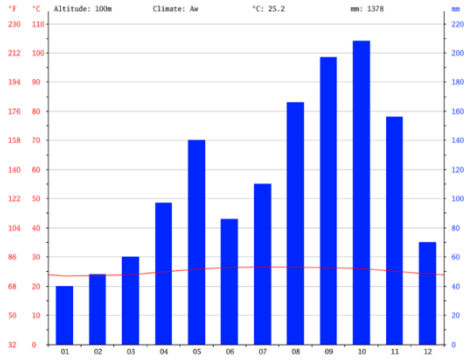


Adapted from website:
https://www.usclimatedata.com/climate.php?location=USPR0058

Figure 4-14. 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 4-15. Climate data for Susúa Commonwealth Forest.

4.2 Habitat Conservation

Habitat conservation is another supportive factor influencing the Puerto Rican harlequin butterfly viability. The establishment of protected areas is the most frequently employed strategy to promote *in situ* biodiversity by conserving natural forested habitat, preventing its conversion to other uses, and reducing anthropogenic threats (Pacarella et al 2000, p. 225; Marcano-Vega 2014, p. 5; Castro-Prieto et al. 2016, p. 1). The Puerto Rican harlequin butterfly range includes several protected lands (e.g., Río Abajo Commonwealth Forest, Guajataca, Río Encantado Natural Protected Area and Maricao and Susúa Commonwealth Forest) (Figures 4-16 and 4-17). While 99.7 percent of the land where the IQC metapopulation occurs is privately owned subjected to development, the other five metapopulation populations occupy areas where substantial portions are managed for conservation (see table 4, below), ranging from 13 percent in Río Encantado; 15 percent in Guajataca; 51 percent in Susúa; 73 percent in Maricao; and 77 percent in Río Abajo (87 FR 73655).

By the mid-20th century, 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 small industry and urban development (Thomlinson et al. 1996, p. 529; Pacarella et al 2000, p. 2018; Rudel et al 2000, p. 386). After 20-60 year of undisturbed forest succession, a significant portion of these abandoned agricultural lands has reached the basal area and species richness found in a subtropical secondary forest (Brown and Lugo 1990, entire, Pacarella et al 2000, p. 217). By 1995, secondary forest cover in Puerto Rico was estimated to be 62 percent due to widespread abandonment of agriculture (Pacarella et al 2000, p. 217). However, more recently, the forest cover in Puerto Rico has shown a decrease from 62 percent to 50.5 percent in 2004 and to 41.7 percent in 2014 (Marcano-Vega 2014, p. 7). In 2014, 83 percent of the estimated forested habitat in Puerto Rico was located on private properties subjected for agricultural, industry, touristic and urban developments (Marcano-Vega 2014, p.5).

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. 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 Puerto Rican harlequin butterfly 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.

On December 1, 2022, the Service published the final rule designating critical habitat for the Puerto Rican harlequin butterfly (87 FR 73655), designating approximately 41,266.0 ac

(16,699.81 ha) in six separate units: IQC, Guajataca, Río Abajo, Río Encantado, Maricao and Susúa; as occupied critical habitat for the Puerto Rican harlequin butterfly (Table 4-1; 87 FR 73657). These critical habitat units were selected based on the best available scientific information on the species, the occurrence of the essential physical and biological features to sustain a species' population, and their adequacy for the conservation of the Puerto Rican harlequin butterfly.

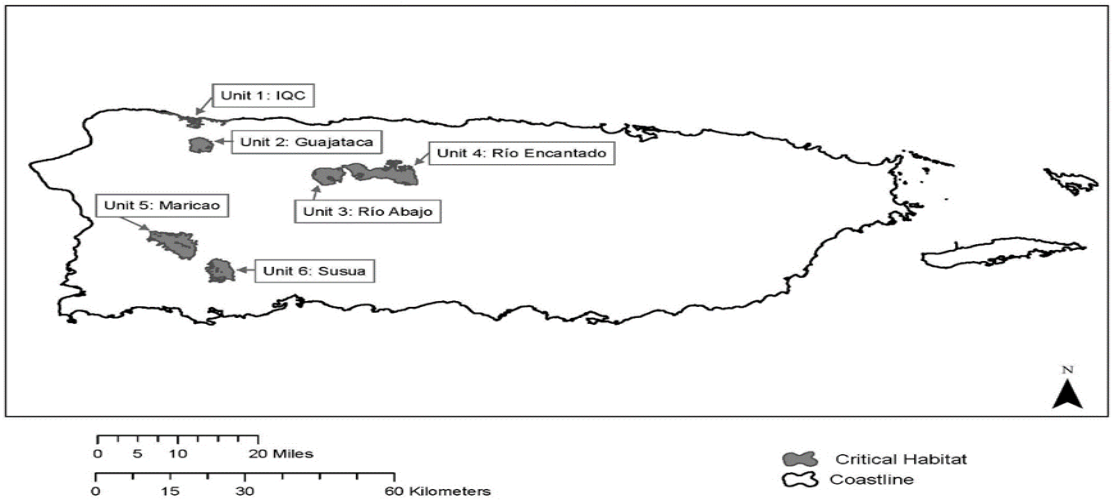


Figure 4-16. Index Map of All Critical Habitat Units for the Puerto Rican Harlequin Butterfly (*Atlantea tulita*), Puerto Rico (87 FR 73655)

Unit 1: IQC

Unit 1 consists of 1,675.7 ac (678.1 ha) located along the northern coastal cliff among the municipalities of Isabela, Quebradillas, and Camuy (IQC), 23 km (15 mi) west of Arecibo. The critical habitat being designated is bound 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 urban developments, State Road PR-2, the Royal Isabela Golf Course, and some deforested areas used for agricultural practices such as cattle grazing. In this unit, 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.

Unit 2: Guajataca

Unit 2 consists of 3,839 ac (1,553.6 ha) south of PR 2, between the municipalities Isabela and Quebradillas, 25 km (15.6 mi) southwest of Arecibo. The critical habitat being designated is bounded on the east by the San Antonio ward in Quebradillas, on the west by State Road PR 446 at Galateo ward in Isabela, on the north by Llanadas ward in Isabela and Cacao ward in Quebradillas, and on the south by Montañas de Guarionex, between the Planas ward in Isabela and Charcas ward in Quebradillas. Fifteen percent of the critical habitat being designated in this unit overlaps Guajataca Commonwealth Forest; an area managed by the DNER for conservation. The other 85 percent is private land subjected to agriculture or rural development.

Unit 3: Río Abajo
Unit 3 consists of 5,939.2 ac (2,403.6 ha) located 14.5 km (9 mi) south of Arecibo. The critical habitat being designated is bound on the east by the Río Grande de Arecibo, on the west by Santa Rosa Ward in Utuado, on the north by Hato Viejo Ward in Arecibo, and on the south by Caguana and Sabana Grande Wards in Utuado. In this unit, all life stages of the species and the host plant have been found at four sites. The Río Abajo Commonwealth Forest, managed for conservation, occupies 77 percent of the unit. The other 23 percent is a mosaic of highways, roads, and private lands subject to agriculture or rural development.

Unit 4: Río Encantado
Unit 4 consists of 12,775.6 ac (5,170.1 ha) located among the municipalities of Arecibo, Florida, and Ciales, 17 km (10.5 mi) southeast of Arecibo. The critical habitat being designated is bound on the east by Hato Viejo Ward in Ciales, on the west by the Río Grande de Arecibo, on the north by Arrozales Ward in Arecibo and Pueblo Ward in Florida, and on the south by the State Road PR 146 along of the Limón Ward in Utuado and Frontón Ward in Ciales. All life stages of the species and the host plant have been found in nine sites. Thirteen percent of the critical habitat being designated is in areas managed by Para La Naturaleza (PLN), a private organization, or by the DNER for conservation. The other 87 percent consists of private lands subject to agriculture or rural developments.

Unit 5: Maricao
Unit 5 consists of 10,854.6 ac (4,392.7 ha) on the west end of the Cordillera Central, among the municipalities of Maricao, San Germán, and Sabana Grande, 16.1 km (10 mi) southeast of Mayagüez. The critical habitat being designated is bound on the east by Tabonuco Ward in Sabana Grande, on the west by Rosario Ward in San Germán, on the north by Pueblo Ward of Maricao, and on the south by the Guamá and Santana Ward of San Germán. All life stages of the species and its host plant have been found at seven sites in the unit. The Maricao Commonwealth Forest, managed for conservation by DNER, comprises 3,996.2 ha (9,874.8 ac) of public land managed for conservation (PA-CAT 2016, http://caribbeanlcc.org/interactive-map). The Commonwealth Forest occupies 72 percent of the unit. The other 28 percent is private land consisting of a mosaic of agriculture, rural developments, and forest.

Unit 6: Susúa
Unit 6 consists of 6,181.9 ac (2,501.8 ha) between the municipalities of Sabana Grande and Yauco, 33.6 km (21 mi) northwest of Ponce. The critical habitat being designated is bound on the east by the State Road PR 371 in Almacigo Alto and Collores Wards in Yauco, on the west by Pueblo Ward in Sabana Grande, on the north by Frailes Ward in Yauco, and on the south by the State Road PR 368 in Susúa Ward in Sabana Grande. All life stages of the species and its host plant have been found at three sites in this unit. The Susúa Commonwealth Forest, managed

by DNER for conservation, occupies 51 percent of the critical habitat being designated in this unit. The other 49 percent is on private lands subjected to agriculture or rural developments.

Table 4-1. Summary of the occupancy of the units, the land ownership, and approximate areas of the designated critical habitat for the Puerto Rican harlequin butterfly (*Atlantea tulita*) (87 FR 73655).

Unit Number/Name	Total Area (acres)	Landowner/ Land Manager(s)	Area (acres)	Percent of ownership	Occupied by the species
Unit 1. IQC	1675.7	Public Land-Municipality	5	0.3	Yes
		Private	1670.7	99.7	
Unit 2. Guajataca	3,839	Public Land-PRDNER	583.5	15	Yes
		Private	3255.5	85	
Unit 3. Río Abajo	5,939.2	Public Land-PRDNER	4544.4	77	Yes
		Private	1394.8	23	
Unit 4. Río Encantado	12,775.6	Private-PLN	1442.6	11.3	Yes
		Public Land-PRDNER	204.8	1.7	
		Private	11128.2	87	
Unit 5. Maricao	10,854.6	Public Land-PRDNER	7,883.1	73	Yes
		Private	2,971.5	27	
Unit 6. Susúa	6,181.9	Public Land-PRDNER	3,171.5	51	Yes
		Private	3,010.4	49	
Total area= 41,266 acres		**Total area (private land) = 24,873.7 (60%) Total area (public land) = 16,392.3 (40%)**			

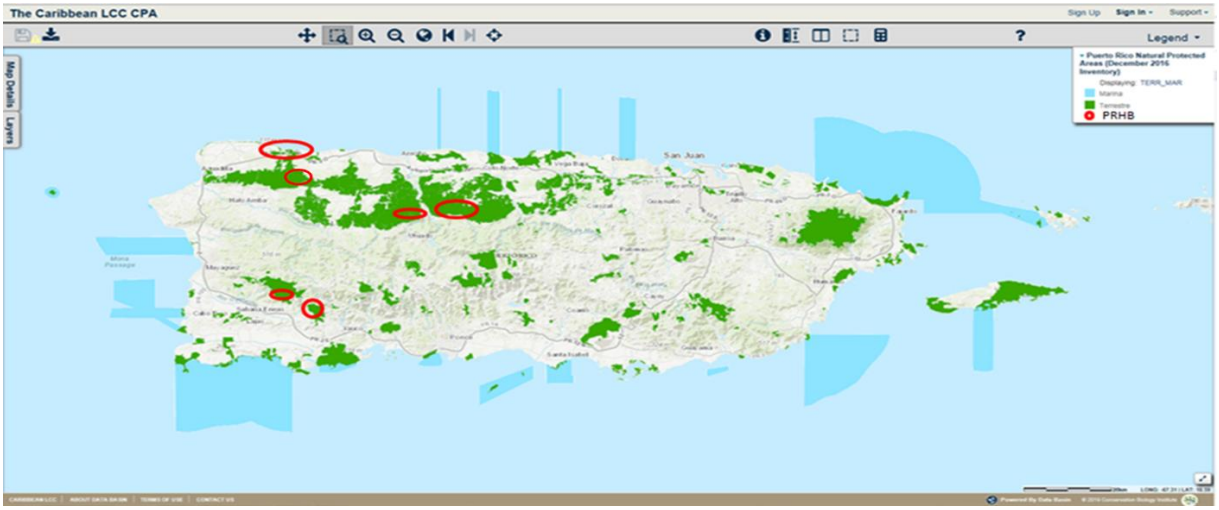


Figure 4-17. Current distribution of the Puerto Rican harlequin butterfly (red circles) in relation with the habitat under protective status (green polygons) in Puerto Rico (PA-CAT 2016, http://caribbeanlcc.org/interactive-map).

4.3 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 Puerto Rican harlequin butterfly viability (Table 4-2, below).

Table 4-2. 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 4-2 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, habitat, 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 Current Condition and Recovery Potential

The Puerto Rican harlequin butterfly was listed as threatened on January 3, 2023 (87 FR 73655), due to threats related to habitat modification and loss, its small populations size, and the results of the analyses concerning projected effects on the species caused by relevant factors (i.e., increasing on urban development rate, use of pesticides, severe atmospheric disturbances, changes in climate conditions) that may negatively influence the continued existence of the Puerto Rican harlequin butterfly in a foreseeable future.

The Puerto Rican harlequin butterfly and its habitat continue to be threatened by the same factors described in the final rule (87 FR 73655). Please see the discussion in the Reason for Listing/Threats Assessment section above. Current and ongoing threats from habitat degradation or loss (Factor A), as well as the application of pesticides (insecticides and herbicides), human induced fires, and climate change (Factor E), contribute to the decline in species abundance and to the fragmentation and isolation of populations. Thus, these factors continue to negatively impact the viability of the Puerto Rican harlequin butterfly. Since listing, the ESA has provided some extra protections to the species and its occupied habitat (Factor D), but the threats of habitat degradation or loss, the application of pesticides, and human-induced fires continues. The best available information indicates that current threats will continue, and the magnitude of the threat by shifts in climate conditions will increase in the foreseeable future.

The Puerto Rican harlequin butterfly has demonstrated some level of resiliency to natural and anthropogenic disturbance in the past. The butterfly has overcome disturbances such as hurricanes, wildfires and habitat loss and fragmentation by vegetation clearance for urban developments and agricultural practices. Nevertheless, the lack of or reduced number of adult individuals can affect population demographics and long-term viability of the species. Therefore, with an overall abundance consisting of less than 100 adult individuals within six scattered populations, the current overall status of the species is in poor condition.

The Endangered Species Act defines as endangered any species that is in danger of extension throughout all or a significant portion of its range. A threatened species is defined as a species that is likely to become endangered within the foreseeable future. The Service believes that, based on the information gathered since the Puerto Rican harlequin butterfly was listed, it still meets the definition of threatened. The threats described in the SSA ver. 1.5 (Service 2019, p.

44), final rule (87 FR 73655) and those discussed in this SSA report indicate that there is a potential for substantial change in the habitat and impacts on individuals that continue to threaten the species to become in danger of extinction in the foreseeable future throughout all its range.

The Puerto Rican harlequin butterfly has an assigned recovery priority number of **11c (**48 FR 43104). This number indicates the species faces a moderate degree of threat and a low recovery potential with conflict. The recovery potential of the species is considered low due to the lack of information on its biology and ecological requirements; hence, the success of management actions with the species is uncertain. The modification and loss of the habitat on which the Puerto Rican harlequin butterfly depends on to survive may limit its recovery potential. Additionally, the recovery of the Puerto Rican harlequin butterfly is, or may be, in conflict with construction or expansion of the State Road PR 120, Calle La Estación and Calle Panoramica roads that provide access between El Merendero, Puente Blanco and Puerto Hermina in Quebradillas, and construction of houses units and touristic development on the cliff along the northern coast of the municipalities of Isabela, Quebradillas and Camuy; therefore, the letter **"c"** was added to the recovery priority.

5.2 Current Resiliency per Metapopulation

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 Puerto Rican harlequin butterfly, 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 the species depend. Moreover, the needs at individual level (suitable forested habitat with adequate food sources, water sources and the host plant, prickly bush 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. Although it is likely that the historical and ongoing habitat degradation and stochastic events have reduced subpopulation connectivity and space for population growth, which reduces resiliency, the persistence of the Puerto Rican harlequin butterfly in the current range indicates that the species can withstand, or at least recover from, some degree of disturbance. On the other hand, the Puerto Rican harlequin butterfly 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 weighted equally, given one (1) point each for a total of 4 points. The overall effect of the population metric was weighted four (4) time higher than each habitat metric (1) because it is a direct measure, given the population metric totals 4 points. Habitat metrics are still important, but it does not give us a direct picture of abundance and therefore does not have more weight that population metric. For each habitat metric were assigned a score of 1, 2, or 3, and for the population metric were 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

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

Habitat occupied by the IQC population is not protected and is at risk of being developed **(1)**. The IQC subpopulations are within 1 kilometer of each other or within the range of forested habitat between populations some forested corridors **(2)**. These subpopulations are 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 in areas with low probability of stochastic events (e.g., fire, severe drought, hurricanes, among others) **(2)**, and there is a relatively high population size **(12)**. Therefore, the IQC population of the Puerto Rican harlequin butterfly is considered to have **moderate resiliency (18)**.

-*Current Resiliency of the Río Abajo Commonwealth Forest Population*

Based on the habitat protection **(3)** the populations or subpopulations are located within 1km range 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 with low probability of stochastic events (e.g., fire, severe drought, hurricanes, among others, among others) **(2)**, and has low population size with a high degree of uncertainty in population size/trends **(4)**, the population of the Puerto Rican harlequin butterfly at Río Abajo Commonwealth Forest is considered to have **moderate resiliency (15)**.

-*Current Resiliency of the Río Encantado Population*

Based on the habitat protection **(2)**, the populations or subpopulations are located within 1km range 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 with low susceptibility to stochastic events (e.g., fire, severe drought, hurricanes, among others) **(2)**, and has low population size with a high degree of uncertainty in population size/trends **(4)**, the population of the Puerto Rican harlequin butterfly at Río Encantado area is considered to have **moderate resiliency (14)**.

-*Current Resiliency of the Guajataca Metapopulation*

Based on the habitat protection **(2)**, the populations or subpopulations are located within 1km range 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 with low susceptibility to

stochastic events (e.g., fire, severe drought, hurricanes, among others) **(2)**, and has low population size with a high degree of uncertainty in population size/trends **(4)**, the population of the Puerto Rican harlequin butterfly at Guajataca area is considered to have **moderate resiliency (14)**.

-*Current Resiliency of the Maricao Commonwealth Forest Population*

Based on the habitat protection **(3)**, the populations or subpopulations are located within 1km range and the habitat between populations or subpopulations is moderated disturbed (forested habitats fragmented by roads and trails) **(2)**, the populations or subpopulations are located in areas subject to periodical vegetation clearing (trails, road and including uses of herbicides along the PR-120) **(1)**; the populations or subpopulations are located in areas subject to stochastic events (e.g. fire, severe drought, hurricanes, among others) **(1)**, and has relatively high population size (more than 20 imagoes and more than 500 caterpillars per ha) and/or growth **(12)**, the population of the Puerto Rican harlequin butterfly at the Maricao Commonwealth Forest is considered to have **moderately high resiliency (19)**.

-*Current Resiliency of the Susúa Commonwealth Forest Population*

Based on the habitat protection **(3)**, the populations or subpopulations are located within 1km range and the habitat between populations or subpopulations is moderated disturbed (forested habitats fragmented by roads and trails) **(2)**, the populations or subpopulations are located in areas subject to periodical vegetation clearing (trails and road) **(1)**; the populations or subpopulations are located in areas subject to stochastic events (e.g. fire, severe drought, hurricanes, among others) **(1)**, and has relatively low population size with a high degree of uncertainty in population trends **(4)**, the population of the Puerto Rican harlequin butterfly at the Susúa Commonwealth Forest is considered to have **low resiliency (11)**.

5.3 Current Overall Resiliency Summary

Presently, of the six populations of the Puerto Rican harlequin butterfly, we classified one as having moderately high resiliency, four with moderate resiliency and one low resiliency (Table 5-2). Our classifications of resiliency count on habitat characteristics and on population size or trend estimates. The population classified as moderate high resiliency (Maricao Commonwealth Forest) occurs in Commonwealth Forest managed for conservation; but the species occurs at edges of or along 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 was classified as moderate resiliency due to it occur in areas where anthropogenic activities may negatively affect the status of the species but has the largest population size known for the species. The populations in Río Abajo Commonwealth Forest, Guajataca and Río Encantado Area were classified as moderate resiliency too because they occur on habitats managed for conservation, surrounded by forested habitat and low probability of been

affected by anthropogenic activities. The populations classified as low (the population at the Susúa Commonwealth Forest) occurs in Commonwealth Forest managed for conservation, the species occur at edges of or along trails and roads where vegetation is frequently removed and herbicide applied, harbor a low population size with high degree of uncertainty in population trends. The overall resiliency is 15 (11 + 19 + 18 + 15 + 14 + 14 = 91 ÷ 6 = 15.1) which is considered moderate resiliency.

Table 5-2. Summary table of the six (6) 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 red, and moderate values are shaded yellow.

Population	**Habitat Metrics**				**Population Metric**	**Resiliency**
	Habitat Protection	Connectivity/ Habitat Fragmentation	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)	<34 percent protected **(1)**	Both **(2)**	High **(1)**	Moderate **(2)**	Relative high population size (more than 20 imagoes and more than 500 caterpillars per ha, and/or growth. **(12)**	**Moderate (18)**
Río Abajo Commonwealth Forest	Most habitat protected (>66 percent) **(3)**	Conectivity **(3)**	Low **(3)**	Moderate **(2)**	Relative low population size (0 to 5 imago and less than 100 caterpillars per ha) or high degree of uncertainty in population size/trends **(4)**	**Moderate (15)**
Río Encantado Area	Some habitat protected (34-66 percent protected) **(2)**	Connectivity **(3)**	Low **(3)**	Moderate **(2)**	Relative low population size (0 to 5 imago and less than 100 caterpillars per ha) or high degree of uncertainty in population size/trends **(4)**	**Moderate (14)**
Guajataca	Some habitat protected (34-66 percent protected) **(2)**	Connectivity **(3)**	Low **(3)**	Moderate **(2)**	Relative low population size (0 to 5 imago and less than 100 caterpillars per ha) or high degree of uncertainty in population size/trends **(4)**	**Modearate (14)**
Maricao Commonwealth Forest	Most habitat protected (>66 percent) **(3)**	Both **(2)**	High **(1)**	High **(1)**	Relative high population size (more than 20 imagoes and more than 500 caterpillars per ha, and/or growth. **(12)**	**Moderately high (19)**
Susúa Commonwealth Forest	Most habitat protected (>66 percent) **(3)**	Both **(2)**	High **(1)**	High **(1)**	Relative low population size (0 to 5 imago and less than 100 caterpillars per ha) or high degree of uncertainty in population size/trends **(4)**	**Low (11)**

5.4 Current Redundancy

The Puerto Rican harlequin butterfly redundancy is influenced primarily by the distribution of populations across spatially heterogeneous environments that would allow the species to persist after a stochastic event, such as hurricanes, severe drought or wildfires. The exact historic distribution (redundancy) of the Puerto Rican harlequin butterfly is unknown, but its present disjoint distribution suggests that they were once more widely distributed across the Island. The species may be subjected to local extirpations from habitat degradation and loss. For the Puerto Rican harlequin butterfly to be viable, there must be adequate redundancy (suitable number, distribution, and connectivity to allow the species to withstand stochastic events) and representation (genetic and environmental diversity to allow the species to adapt to changing environmental conditions). Redundancy improves with increasing numbers of populations distributed across the species range, and connectivity (either natural or human-facilitated) allows connected populations to "rescue" each other after catastrophes.

Redundancy for the Puerto Rican harlequin butterfly, a narrow ranging endemic, is inherently low. Its reduction could be attributed to wide scale habitat destruction and other factors that have isolated and extirpated populations. Currently, with six very small populations and only one of those considered to have moderately high resilience, the species is not well buffered against the effects of stochastic events. Stochastic events that could affect single or multiple Puerto Rican harlequin butterfly populations include but are not limited to hurricanes (multiple populations), increase of abundance of predators (single or multiple populations), disease outbreaks, use of pesticides (single or multiple populations) and fires (single populations). Moreover, conversion of forested habitats for agriculture and urbanization could continue affecting existing populations and the habitat on 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.

Current distribution, number of populations and subpopulations, and population index for the Puerto Rican harlequin butterfly is discussed in Chapter 3 (above).

5.5 Current Representation

The Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly populations. Currently, representation appears to be moderate to high because the Puerto Rican harlequin butterfly occurs in four ecological settings or life zones. Thus, the Puerto Rican harlequin butterfly seems to have the capability to adapt to different landscapes if the fundamental needs for nesting (host plant) and foraging are met.

5.6 Summary of Puerto Rican harlequin butterfly condition based on the 3Rs

There is sufficient information to conceptualize and estimate the condition of the 3Rs for the Puerto Rican harlequin butterfly (Figure 5-1). Currently Puerto Rican harlequin butterfly populations range wide have representation in two geographic regions and four life zones. There are six metapopulations that serve as a measure of species redundancy. One of those metapopulation has moderately high resiliency, four have moderate resiliency, and the remaining one population have low resiliency.

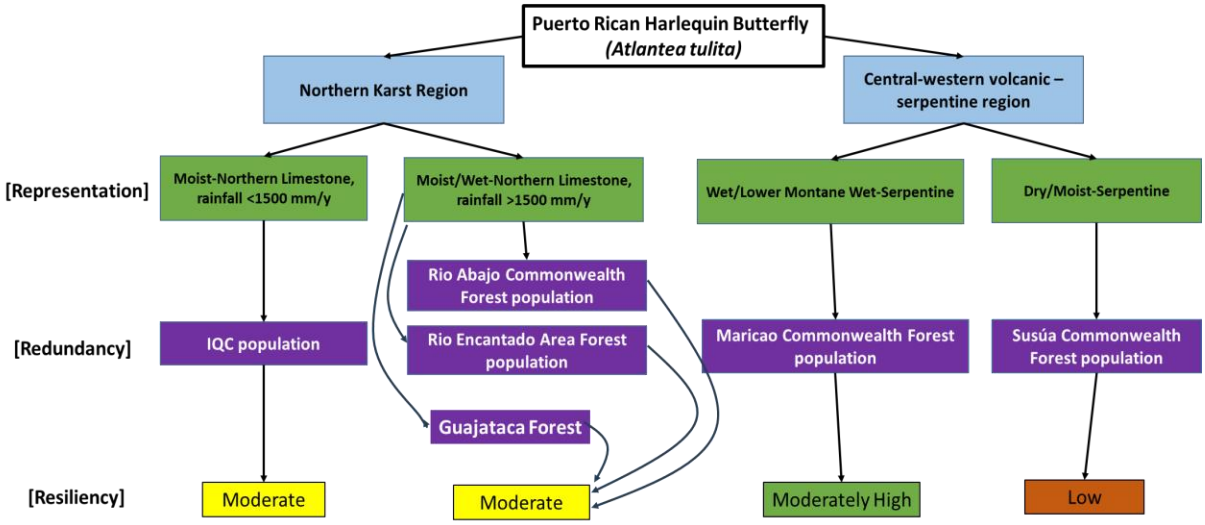


Figure 5-1. Basic conceptual model showing how the Puerto Rican harlequin butterfly is represented (life zones), its redundancy (populations) and its resiliency.

CHAPTER 6- FUTURE CONDITION SCENARIOS

6.1 Introduction

In this chapter, we describe our analysis of the future conditions for the Puerto Rican harlequin butterfly. 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 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 Puerto Rico harlequin butterfly 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 Puerto Rican harlequin butterfly 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 shifts in climate conditions. 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 Puerto Rican harlequin butterfly 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 environmental stochasticity. 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 Puerto Rican harlequin butterfly.

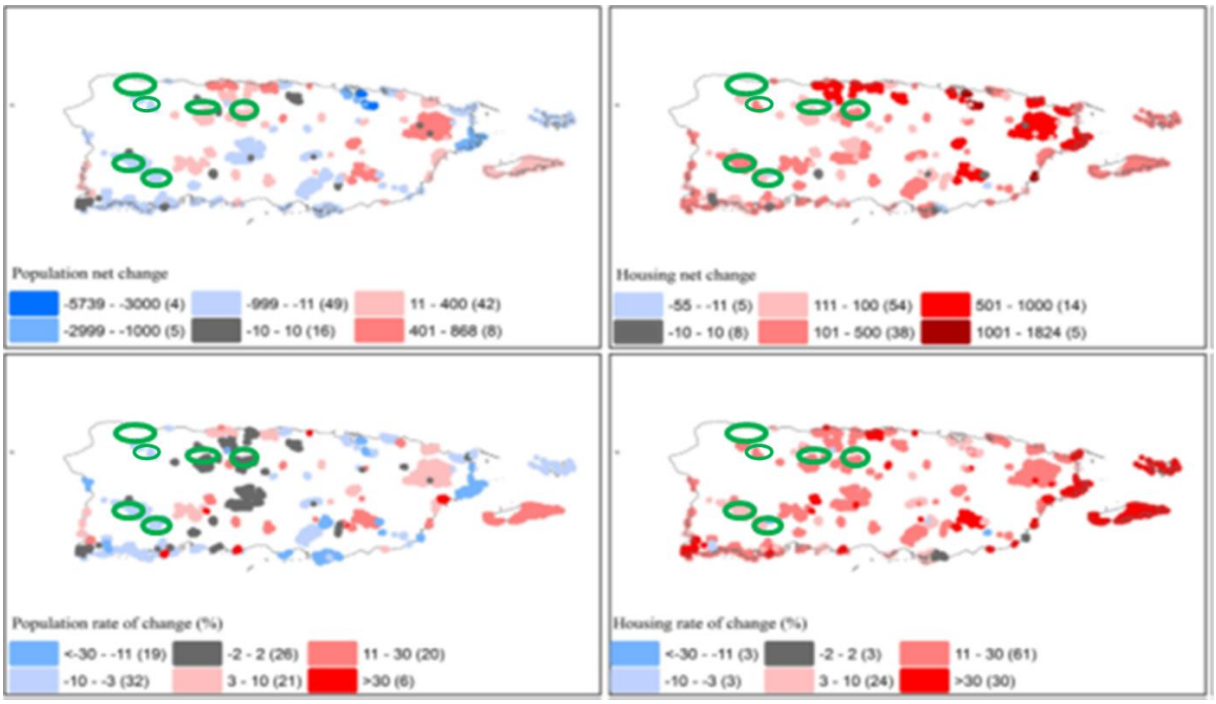


Figure 6-1. Spatial patterns of housing and human population changes within 1 km of protected areas. The number of protected areas in each class is shown in parentheses (Castro-Prieto 2017, p. 477). Green circles indicate the location of the Puerto Rican harlequin butterfly populations in Puerto Rico.

Table 6-1. Projected housing net change within one (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.

1 Kilometer buffer zone around Puerto Rican harlequin butterfly population			
Population	**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
Guajataca	18 percent per decade	9 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 changes in climate conditions.

- *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).

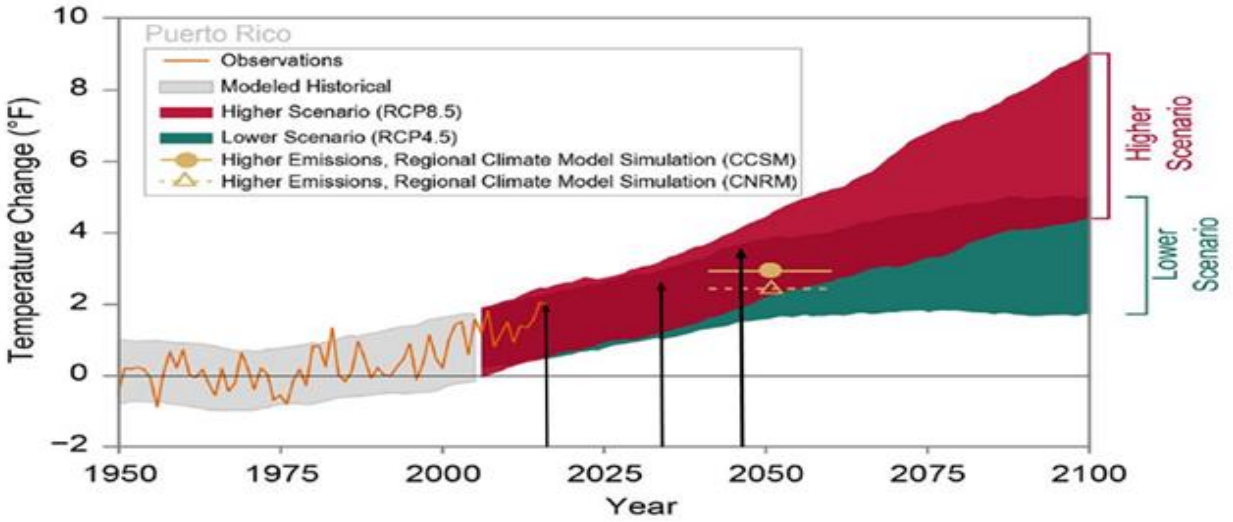


Figure 6-2. Observed and projected temperature changes are shown compared to the 1951-1980 average. Observed data are for 1950-2017, and the range of model simulations for the historical period is for 1950-2005. The range of projected temperature changes from global climate models is shown for 2006-2100 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.

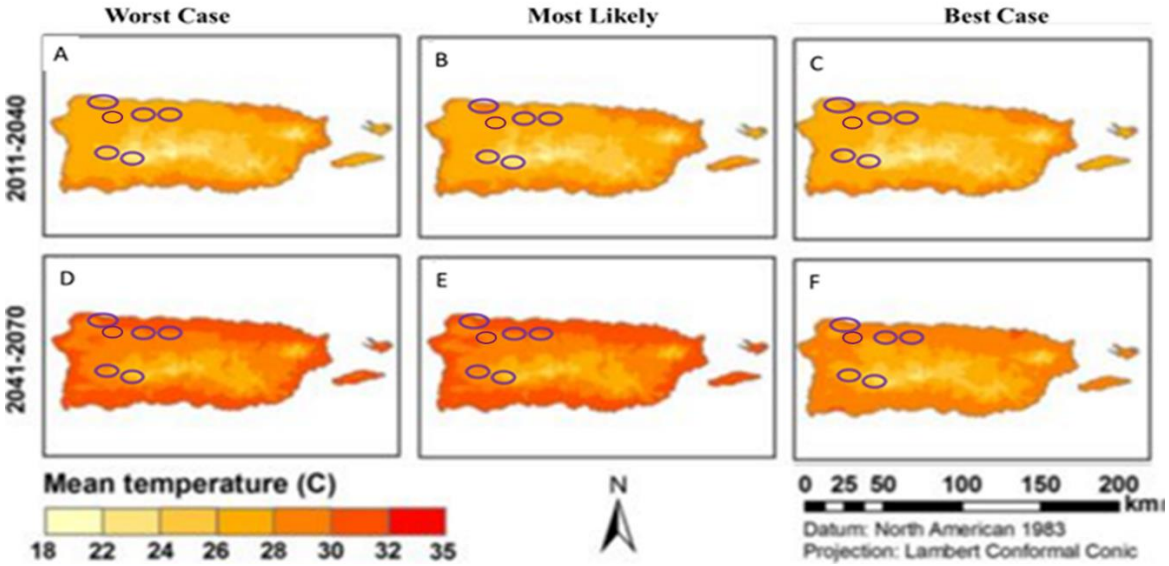


Figure 6-3. Temperature projections for Puerto Rico under three general circulation models (GCM) scenarios (Henareh et al. 2016, p. 277). Purple circles indicate the locations of the Puerto Rican harlequin butterfly populations. Figures A, B and C show current condition and the projection for 20 years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

- *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, most 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).

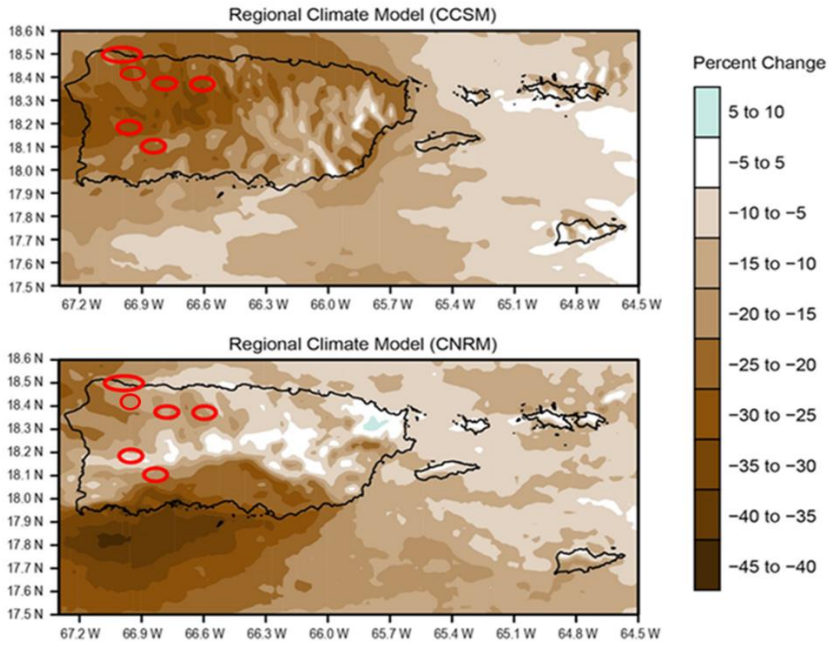


Figure 6-4. Projected Precipitation Change for the U.S. Caribbean. This figure shows the projected percent change in annual precipitation over the U.S. Caribbean region for the period 2040-2060 (lower figure) compared to 1985-2005 (upper figure) based on the results of two regional climate model simulations. 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 Puerto Rican harlequin butterfly populations in Puerto Rico.

While we currently do not know the maximum thermal tolerance of the Puerto Rican harlequin e butterfly, 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 Puerto Rican harlequin butterfly occurs in environments with annual average maximum temperatures of 82-90ºF (28-32ºC). However, based on our future climate projections (Table 6-2), 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 Puerto Rican harlequin butterfly populations by 2045. Although the effects of this temperature increase on reproductive success of the Puerto Rican harlequin butterfly remain uncertain, the cited studies suggest that Puerto Rican harlequin butterfly reproduction may be adversely affected. Moreover, given that egg-laying and subsequent larval growth of the Puerto Rican harlequin butterfly is closely associated with new leaf growth of prickly bush triggered by the onset of the rainy season (ca. May-June; Figures 3-10), any future climatic aberrations which disrupt or reduce such rains will also likely have a detrimental effect on Puerto Rican harlequin butterfly 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.

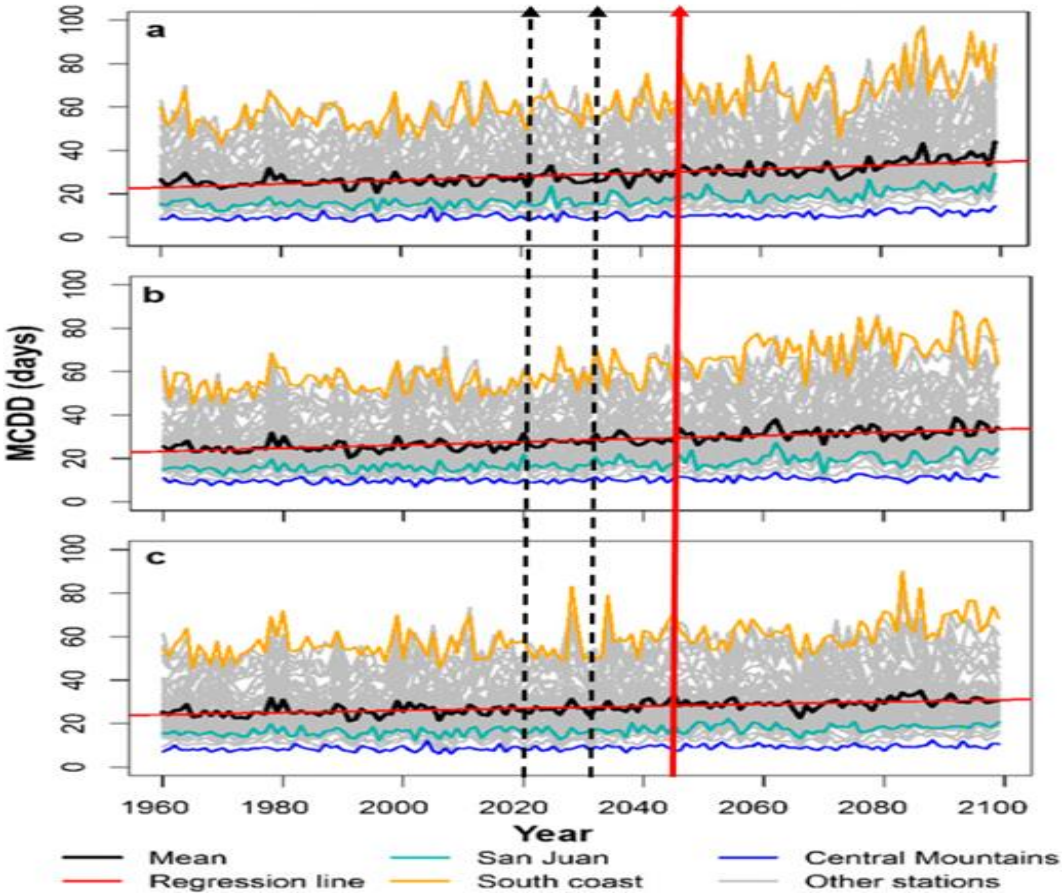


Figure 6-5. Maximum number of consecutive dry days (MCDD) in Puerto Rico. Black arrows represent present time (2020) and 10 years into the future. Red arrow indicates MCDD at 25 years into the future (2045). Panel A represents "worst case scenario"; B represents "most likely scenario"; C represents "best 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

- *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 Puerto Rican harlequin butterfly, 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 Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly to adapt to such conditions is expected to be reduced due to the current small number of populations and individuals.

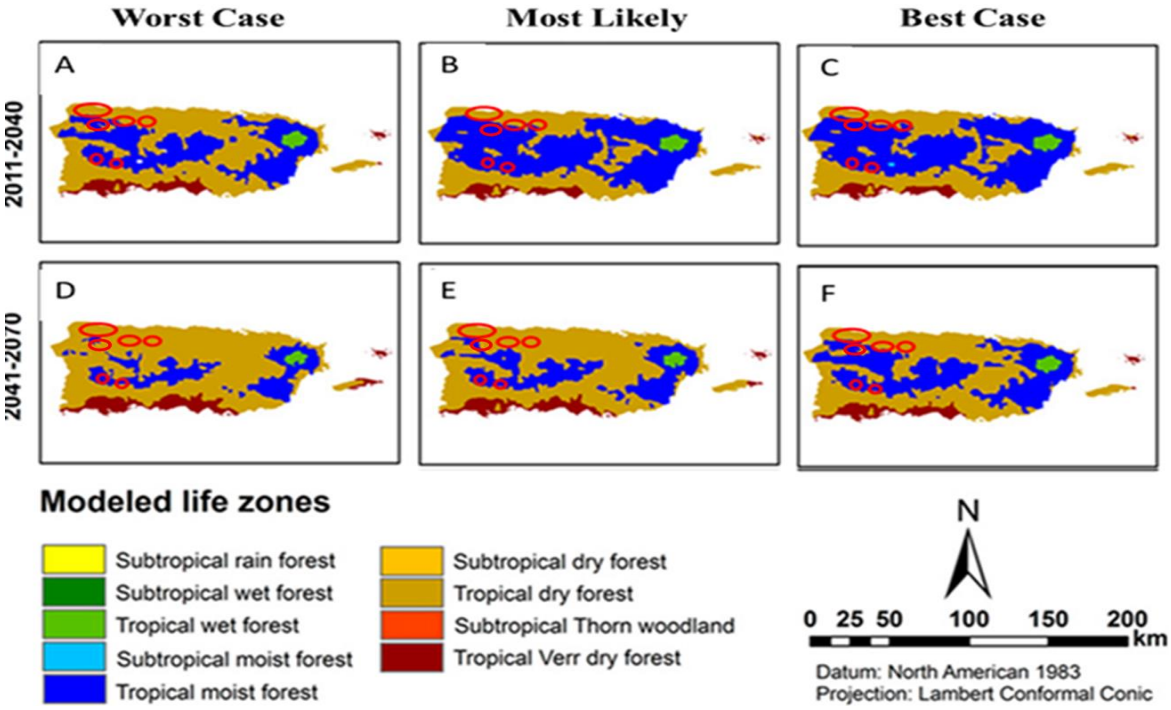


Figure 6-6. Projected life zones from the average of all models under the three future scenarios. Red circles indicate currently known populations of Puerto Rican harlequin butterfly. Figures A, B and C show current condition; Figures D, E and F show the projection at 25 years (2045) and beyond. Adapted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

- 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 Puerto Rican harlequin butterfly and its habitat.

Long-term viability will require resilient populations in locations that are protected from long-term catastrophic but permanent effects of shifting climate conditions (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 Puerto Rican harlequin butterfly 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.

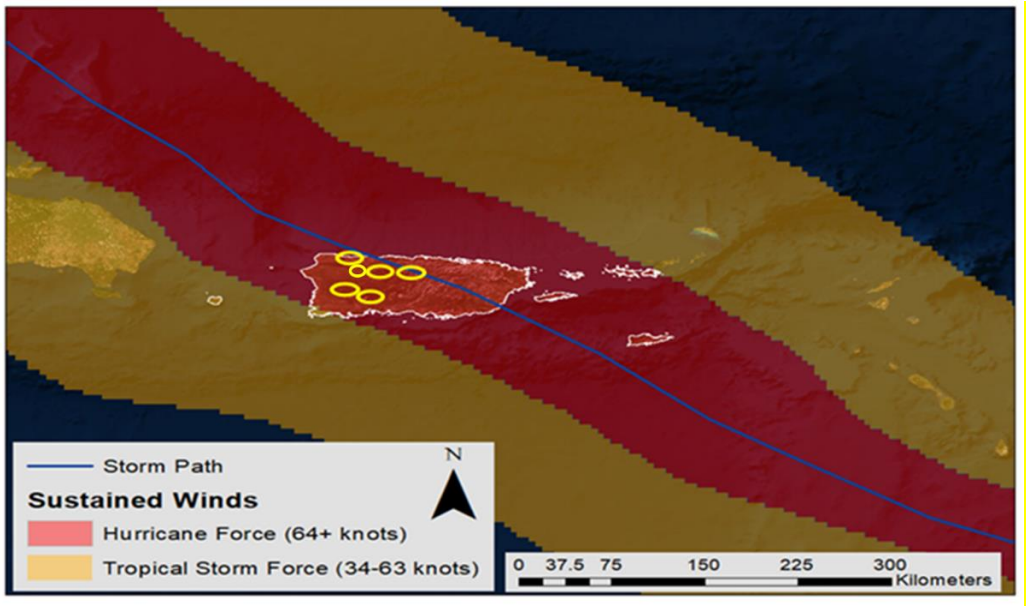


Figure 6-7. Path and wind speed of Hurricane Maria in September 2017. Puerto Rico and the US Virgin Islands are outlined in white, and the approximate range of the Puerto Rican harlequin butterfly is circled in yellow. (Data accessed from National Hurricane Center, National Oceanic and Atmospheric Administration,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

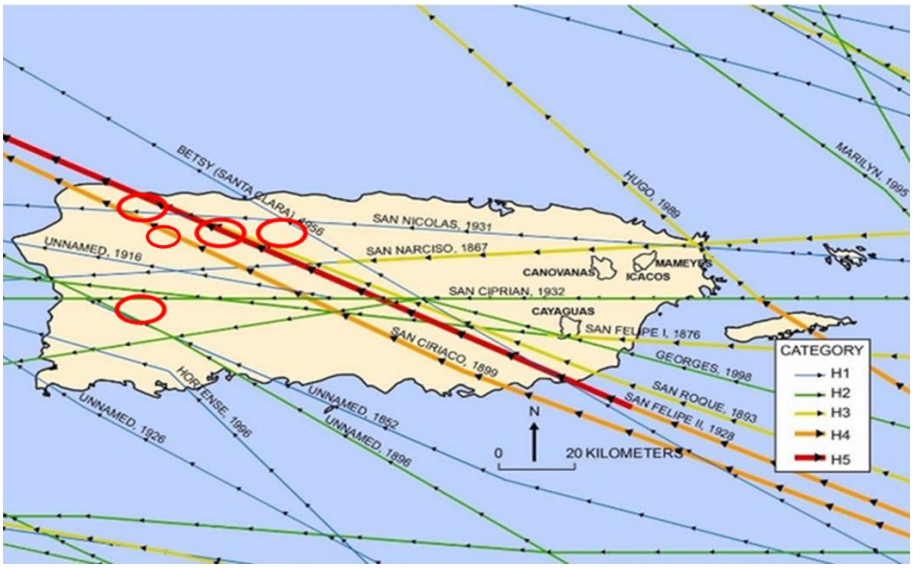


Figure 6-8. A sample of historical hurricanes that have struck Puerto Rico (USGS, public domain https://www.usgs.gov/media/images/puerto-rico-hurricanes-map), red circles identify the current locations of the Puerto Rican harlequin butterfly populations (Adapted from Feng et al. 2018, p. 3).

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 population metric is less reliable because here it is a reflect condition of the habitat and is not a direct measure as is in current condition. The habitat metrics are the drivers that may promote changes in future population (unless the population is so low that loss of individuals significantly affects population metric). Therefore, habitat metrics had more weight that population metric in the overall weight.

Habitat metrics is represented by four (4) measures, representing the 66 percent of the total score and population metrics is representing the 33 percent for the future conditions. For each habitat metric were assigned a score of one (1), two (2), or three (3), and for the population metric were assigned a score of two (2), four (4), or six (6), as described below in Table 6-3. Each habitat measure was weighted equally; with the overall effect that habitat (4 metrics) was weighted two (2) times higher than the population metric (2). As it was not possible to quantify with any reliability the future population sizes or trends under any future scenarios, we therefore assumed “no change” from current conditions for this metric for “Best Case Scenario”, and assigned a score reduction of “-1” (e.g., from 0 to -1) for current condition population scores for “Most Likely Scenario” and a score reduction of “-2” for current condition population scores for “Worst

Case Scenario." We used this approach as a reasonable means of accounting for expected (albeit unquantifiable) adverse effects of predicted reductions in habitat metrics on associated Puerto Rican harlequin butterfly reproduction and survival.

Table 6-3. Description of how habitat and population factors were scored to determine the Puerto Rican harlequin butterfly population future 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 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

Predicted 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 and changes in climatic conditions (Low, Moderate, or High)	Population Size/Trend (Description)	
Isabela, Quebradillas and Camuy (IQC)	Risk of Development **(1)**	Habitat fragmented/ low conectivity **(1)**	High **(1)**	High (1)	Relative low population size (0 to 5 imago and less than 100 caterpillars per ha) or high degree of uncertainty in population size/trends **(2)**	**Low (6)**
Río Abajo Commonwealth Forest	Most habitat protected (>66 percent) **(3)**	Conectivity **(3)**	Low **(3)**	**High (1)**	Extirpated	**Extirpated**
Río Encantado Area	Some habitat protected (34-66 percent protected), some at risk of being developed **(2)**	Both **(2)**	Moderate **(2)**	High **(1)**	Extirpated	**Extirpated**
Guajataca	Some habitat protected (34-66 percent protected) **(2)**	**Both (2)**	**Moderate (2)**	High **(1)**	Extirpated	**Extirpated**
Maricao Commonwealth Forest	Most habitat protected (>66 percent) **(3)**	Both **(2)**	High **(1)**	High **(1)**	Relative low population size (0 to 5 imago and less than 100 caterpillars per ha) or high degree of uncertainty in population size/trends **(2)**	**Moderately low (8)**
Susúa Commonwealth Forest	Most habitat protected (>66 percent) **(3)**	Both **(2)**	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 and changes in climatic conditions (Low, Moderate, or High)	Population Size/Trend (Description)	
Isabela, Quebradillas and Camuy (IQC)	Risk of Development **(1)**	Habitat fragmented/ low conectivity **(1)**	High **(1)**	High (1)	Relative moderate population size (15 to 20 imago and less than 100 to 500 caterpillars per ha) **(4)**	**Low (8)**
Río Abajo Commonwealth Forest	Most habitat protected (>66 percent) **(3)**	Connectivity **(3)**	Low **(3)**	Moderate **(2)**	**Extirpated**	**Extirpated**
Río Encantado Area	Some habitat protected (34-66 percent protected), some at risk of being developed **(2)**	Both **(2)**	Low **(3)**	Moderate **(2)**	**Extirpated**	**Extirpated**
Guajataca	Some habitat protected (34-66 percent protected), some risk of being developed **(2)**	**Both (2)**	**Moderate (2)**	Moderate **(2)**	Extirpated	**Extirpated**
Maricao Commonwealth Forest	Most habitat protected (>66 percent) **(3)**	Both **(2)**	Moderate **(2)**	High **(1)**	Relatively moderate population size (15 to 20 imago and 100 to 500 caterpillars per ha) or high degree of uncertainty in population size/trends **(4)**	**Moderately (11)**
Susúa Commonwealth Forest	Most habitat protected (>66 percent) **(3)**	Both **(2)**	Moderate **(2)**	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 and changes in climatic conditions (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 caterpillars per ha), and/or growth **(6)**	**Moderate (12)**
Río 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 caterpillars per ha) or high degree of uncertainty in population size/trends **(2)**	**Moderate (13)**
Río Encantado Area	Some habitat protected (34-66 percent protected), some at risk of being developed **(2)**	Connectivity **(3)**	Low **(3)**	Moderate **(2)**	Relatively low population size (0 to 5 imagoes and less than 100 caterpillars per ha) or high degree of uncertainty in population size/trends **(2)**	**Moderate (12)**
Guajataca	Some habitat protected (34-66 percent protected), some risk of being developed **(2)**	Connectivity **(3)**	Low **(3)**	Moderate **(2)**	Relatively low population size (0 to 5 imagoes and less than 100 caterpillars per ha) or high degree of uncertainty in population size/trends **(2)**	**Moderate (12)**
Maricao Commonwealth Forest	Most habitat protected (>66 percent) **(3)**	Both **(2)**	Moderate **(2)**	High **(1)**	Relatively high population size (more than 20 imagoes and more than 500 caterpillars per ha), and/or growth **(6)**	**Moderately high (14)**
Susúa Commonwealth Forest	Most habitat protected (>66 percent) **(3)**	Both **(2)**	Moderate **(2)**	High **(1)**	Relatively low population size (0 to 5 imagoes and less than 100 caterpillars per ha) or high degree of uncertainty in population size/trends **(2)**	**Moderately low (10)**

6.4.1 Future Resiliency

Future resiliency of four of the six Puerto Rican harlequin butterfly populations is expected to decline to "Extirpated" under our "Most Likely" and "Worst Case" scenarios (Table 6-7). Collectively, these four populations represent approximately 66 percent of the entire known Puerto Rican harlequin butterfly 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 six populations persist at levels comparable to current conditions (Table 6-7).

Table 6-7. Summary of Puerto Rican harlequin butterfly population resiliency under current and future scenarios.

Population	Current (2025)	Worst Case	Most Likely	Best Case	Approximate percentage of total population [1]
Isabela, Quebradillas and Camuy (IQC)	Moderate (18)	Low (6)	Low (8)	Moderate (12)	50
Río Abajo Commonwealth Forest	Moderate (15)	Extirpated	Extirpated	Moderate (13)	<5
Río Encantado Area	Moderate (14)	Extirpated	Extirpated	Moderate (12)	<5
Guajataca Forest	Moderate (14)	Extirpated	Extirpated	Moderate (12)	<5
Maricao Commonwealth Forest	Moderately high (19)	Moderately low (8)	Moderate (11)	Moderately high (14)	20
Susúa Commonwealth Forest	Low (11)	Extirpated	Extirpated	Moderately low (10)	15

[1]Based on most recent (Barber 2018) field counts of imagoes (adult individuals) and the SSA ver. 1.5 (Service 2019).

6.4.2 Future Representation

According to our "Most Likely" and "Worst Case" scenarios, all areas and life zones which currently harbor Puerto Rican harlequin butterfly populations are expected to become drier and warmer, with some (i.e., Guajataca Forest, Río Abajo and Río Encantado) progressing from tropical moist forest to tropical dry forest (Figure 61). Under these scenarios, and with only two 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 (4/5) Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly 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 shifting climate conditions. The continuing development – residential, commercial and tourist – both within and adjacent to areas currently occupied by Puerto Rican harlequin butterfly will most likely increase over this time, 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 Puerto Rican harlequin butterfly biological and ecological needs and requirements. However, widespread and continuing lax application and enforcement of existing regulations that aim to protect Puerto Rican harlequin butterfly 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 Puerto Rican harlequin butterfly. 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 period. While the full ecological effects of these changes on the Puerto Rican harlequin butterfly 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 prickly bush populations) will have negative effects on the Puerto Rican harlequin butterfly.

In summary, at the end of our predictive time horizon (year 2045) at least four of the current six Puerto Rican harlequin butterfly 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, Guajataca, Río Encantado, and Susúa, representing approximately 43 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 Puerto Rican harlequin butterfly population will be substantially greater than 43 percent, although impossible to accurately quantify at the current time.

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