# Puerto Rican Crested Toad (Peltophryne lemur)



Photo by: Carlos Pacheco, USFWS biologist, 2012

# 5-Year Review: Summary and Evaluation

U.S. Fish and Wildlife Service Southeast Region Caribbean Ecological Services Field Office Boquerón, Puerto Rico

# 5-YEAR REVIEW

# Puerto Rican crested toad / Peltophryne lemur

#### I. GENERAL INFORMATION

#### A. Methodology used to complete the review:

On September 21, 2007, the U.S. Fish and Wildlife Service (hereafter the Service) published a notice in the *Federal Register* (72 FR 54061) announcing the 5-year review of *Peltophryne lemur*, commonly known as the Puerto Rican crested toad (PRCT), and requesting new information concerning the biology and status of the species. A 60-day comment period was opened. No information on the PRCT was received from the public during that public comment period.

This 5-year review was prepared by the PRCT lead Service recovery biologist and summarizes new information that the Service has gathered in the PRCT file since the recovery plan for the species was signed on August 7, 1992 (USFWS 1992). The source of information used for this review included the final listing rule for the species, the recovery plan for the species, peer-reviewed literature, personal communications with qualified biologists and experts on this species, and unpublished reports from field observations and recovery activities conducted by Service biologists.

We sent this document for peer review to experts at the Puerto Rico Department of Natural and Environmental Resources (PRDNER), Association of Zoos and Aquariums (AZA; formerly known as AZAA), and other local experts on the species. Comments and recommendations received were evaluated and incorporated in the 5-year review accordingly (See Appendix A). Therefore, we believe to have included the best available information on the species in this review.

#### **B.** Reviewers

**Lead Region:** Kelly Bibb, Southeast Regional Office, Atlanta, GA, (404) 679-7132.

**Lead Field Office:** Carlos Pacheco, Caribbean Ecological Services Field Office, Boquerón, Puerto Rico, (787) 851-7297, extension 221.

# C. Background

- **1. Federal Register Notice citation announcing initiation of this review:** September 21, 2007; 72 FR 54061.
- **2. Species Status:** 2014, improving. Since the PRCT was listed in 1987, the Service, PRDNER, AZA, and other Partners have continuously managed both the wild and captive populations. These activities include: protection and enhancement of the species habitat and breeding ponds, removal of competitors and predators from breeding ponds and adjacent

habitats, maintaining a healthy captive breeding population, evaluation of new introduction sites, design and construction of rearing and breeding ponds, release of tadpoles and toadlets, and education and outreach, among other recovery activities stated in the species recovery plan and recommended by the species experts. From 2003 to present, various governmental and non-governmental organizations have been working with the Service towards the recovery of the PRCT. The group, named the PRCT Working Group (Group), includes representatives of the Service, PRDNER, AZA, Puerto Rico National Park Company (PRNPC), Para La Naturaleza (PLN, formerly known as the Puerto Rico Conservation Trust), researchers, scientists, local NGO's, universities, educators, private landowners, community groups and volunteers. This group is led by the Service. Collectively, some of the priority recovery actions achieved are: (1) over 250,000 tadpoles and 520 toadlets released over the past 13 years on six sites managed for conservation in Puerto Rico: three sites in the southern karst region and three sites in the northern karst region; (2) the species has been introduced successfully at Manglillo Grande in the Guánica Commonwealth Forest (GCF), where adult individuals and breeding events have been documented since releases; (3) PRCT adult and sub-adult individuals (female and males older than one year of age) are frequently sighted at the private natural reserve El Tallonal in Arecibo, at Gabia Farm (PRDNER) in Coamo, at Río Encantado (PLN) in Ciales, at La Esperanza in Manatí (PLN), and at El Convento in Guayanilla (PLN); though no successful breeding events have been documented yet; (4) a new natural breeding site for the species was discovered at Punta Ventana (private land) in Guayanilla; (5) the PRCT was rediscovered in a private land at Ciénaga Ward, Yauco; and (6) the captive program has grown to over 900 toads across more than 31 zoological institutions in the U.S. and Canada. Additionally, the PRCT was included as one of the key species in a landscape-level project within the Service's Strategic Habitat Conservation Plan for Puerto Rico. This project includes actions needed for enhancement of the species habitat (i.e., construction of breeding ponds, reforestation) in the northern and southern karst regions in Puerto Rico.

**3. Recovery Achieved:** 3 (3 = 51-75%) of species' recovery objectives achieved.

#### 4. Listing History

**Original Listing** 

FR notice: 52 FR 28828 Date listed: August 4, 1987

Entity listed: species Classification: threatened

**5. Associated rulemakings**: None

#### 6. Review History:

The August 4, 1987 final rule (52 FR 28828) and the Recovery Plan for the Puerto Rican crested toad (hereafter the recovery plan) approved on August 7, 1992 (USFWS 1992) are the most comprehensive analyses for the species and are used as the reference point documents for this 5-year review.

The PRCT, commonly known in Spanish as *sapo concho puertorriqueño*, is the only native bufonid of Puerto Rico and Virgin Islands. The PRCT was first discovered by George Latimer in an unidentified location in Puerto Rico (Cope 1868, Stejnerger 1902). Subsequently, the species was collected from eight scattered localities in Puerto Rico and one in Virgin Gorda, Britsh Virgin Island (BVI; Figure 1, see p. 15) (USFWS 1992). The PRCT has been described as a rare species throughout its entire range because the low number of collections (Miller 1985, Service 1992). From 1868 to 1931, a total of 28 specimens were collected and no additional collection came to light in a period of 34 years (García-Díaz 1967). This led herpetologists to believe that the species had become extinct. In 1966, the species was rediscovered by Dr. Julio García Díaz at Cotto Ward in Isabela, Puerto Rico (García-Díaz 1967, Estremera 1990). This PRCT population falls within the northern karst region in Puerto Rico. Later in July 1984, Moreno and Canals (1985) discovered two breeding populations in southern karst region in Puerto Rico; one at the GCF and another at Ciénaga in Barinas Wards, municipality of Yauco.



Figure 1. Map of the Puerto Rican Shelf indicating localities from which the Puerto Rican crested toad has been collected and was known to occur at the time the species recovery plan was approved in 1992 (USFWS 1992).

Since its reappearance, the Service, the PRDNER and AZA have been working together for the protection and conservation of the crested toad. The PRCT was included in the Species Survival Plan (SSP) by AZA in 1984, being the first amphibian to be included in the program because of its limited distribution (Johnson 1990, Service 1992).

In the August 4, 1987 final rule (52 FR 28828), the Service reviewed the best scientific and commercial information available, analyzed the five listing factors, and listed the PRCT as

threatened. The Service identified Factor A (present or threatened destruction, modification, or curtailment of its habitat or range), and Factor E (other natural or manmade factors affecting its continued existence) as the main threats for the species. The recovery plan included the description of the species and information about its distribution, abundance, habitat characteristics, reproductive biology, analysis of the five listing factors, and conservation measures. At the time the recovery plan was approved, the PRCT was known only from two populations; one of approximately 2,000 toads in southern Puerto Rico within the GCF and another population of approximately 25 to 50 toads in the north coast at Quebradillas (Service 1992).

The Service conducted a 5-year review for the Puerto Rican crested toad in 1991 (56 FR 56882). In this review, the status of many species was simultaneously evaluated with no indepth assessment of the five factors or threats as they pertain to the individual species. The notice stated that the Service was seeking any new or additional information reflecting the necessity of a change in the status of the species under review. The notice indicated that if significant data were available warranting a change in a species' classification, the Service would propose a rule to modify the species' status. No new information or additional data was received. Therefore, no change in the PRCT's listing classification was found to be appropriate.

Each year the Service reviews and updates listed species information to benefit the required Recovery Report to Congress. Through 2013, we did a recovery data call that included showing status recommendations like "Improving" for this amphibian. We continue to show that species status recommendation in 5-year reviews. The most recent evaluation for this amphibian was completed in 2015.

7. Species' Recovery Priority Number at start of review (48 FR 43098): <u>2c</u>. At the time of listing, the PRCT was determined to be a species with a high degree of threat, but a high recovery potential. Additionally, the recovery of the species was considered to have, or may have conflicts with construction or other development projects or other forms of economic activity.

#### 8. Recovery Plan:

Name of plan: Recovery Plan for the Puerto Rican Crested Toad (*Peltophryne lemur*)

**Date issued:** August 7, 1992

#### II. REVIEW ANALYSIS

- A. Application of the 1996 Distinct Population Segment (DPS) policy
- 1. Is the species under review listed as a DPS? No
- 2. Is there relevant new that would lead you to consider listing this species as a DPS in accordance with the 1996 policy? No

#### **B.** Recovery Criteria

1. Does the species have a final, approved recovery plan containing objective, measurable criteria? Yes. The measurable criteria included in the plan to delist the species established the number of populations and individuals needed for recovery. Although these criteria could be measurable, the number of individuals per population included in the criteria was based on anecdotal information and not on reliable population estimates.

#### 2. Adequacy of recovery criteria

- **a.** Do the recovery criteria reflect the best available and most up-to-date information on the biology of the species and its habitat? No. The PRCT has a final approved recovery plan (USFWS 1992), but it is outdated. At the time the plan was approved, information regarding species' biology, distribution, habitat requirements and life history was limited.
- **b.** Are all of the five listing factors that are relevant to the species addressed in the recovery criteria? When the recovery plan was approved, the species was threatened by Factor A and Factor E. The recovery plan also stated that Factor C could be considered as a threat to the species, but its magnitude and imminence were speculative at that time. Although the recovery plan includes the five listing factor analysis, they were not addressed fully by the recovery criteria. As we look at possible revisions to our recovery plan, we will consider more objective criteria to address the threats to this species.
- 3. List the recovery criteria as they appear in the recovery plan, and discuss how each criterion has or has not been met, citing information.

The plan states that delisting of the PRCT will be considered when at least three wild populations in the north and three wild populations in the south are established and maintained. The plan specifies that each wild population should consist of 1,500 to 2,000 toads and that at least five captive populations of 300 toads each should be established.

Based on the gathered information, the recovery criteria have been partially accomplished as follows:

At present, one large wild population of the PRCT occurs in southern Puerto Rico. This population is comprised by three reproductive sub-populations: one at the GCF, one at Ciénaga wetland in Yauco, and another at Punta Ventana pond in Guayanilla; all located less than 2.5 miles (4.0kilometers) from each other. Additionally, efforts to establish new PRCT populations along the historical range of the species in Puerto Rico are ongoing since 1992. Over 312,000 tadpoles and 1,546 toadlets of PRCT have been released at six locations in Puerto Rico (D. Barber, AZA, 2014 unpubl. data; Figure 3, USFWS, 2014, unpubl. data). As a result of this reintroduction effort, the species has been established successfully at Manglillo Grande in GCF, with first documented reproduction events occurring in 2003 (M. Canals, former GCF Manager, PRDNER, 2003, unpubl. data). Additionally, we are in the process of placing PRCT in five other sites; three in the northern karst (i.e., El Tallonal, Río Encantado, and La Esperanza), and other two in the southern karst (i.e., Gabia Farm and El Convento).

Currently, adult crested toads (females and males) have been observed in these five reintroduction sites, but no successful reproductive events have been reported yet. Additionally, in 1989, the Service, PRDNER and AZA released 800 PRCT toadlets at the Cambalache Commonwealth Forest in northern Puerto Rico (USFWS 1992), but the success of this effort is not known because the species has not been surveyed at this site.

The criterion based on number of individuals per population should be reconsidered. To be able to determine the number of PRCTs in a wild population is a daunting task, particularly because the cryptic behavior of the species makes it difficult to detect (C. Pacheco, USFWS, pers. obs.) Consequently, estimating its abundance and population densities is challenging if biological studies are not carefully conducted. Presently, the abundance of PRCT per natural populations is expressed in terms of number of toads counted per reproductive events (M. Canals, former GCF Manager, PRDNER, 2008, unpubl. data), and in terms of number of toads sighted per night search (Silva-Lee, SFWF, 2012, unpubl. data; C. Pacheco (USFWS) and D. Barber (AZA), 2014, unpubl. data). At the re-introduction sites, the population estimates are based on number of toads observed per night search and number of individuals (tadpoles and toadlets) released at each sites. Hence, toad counts may fluctuate according to the intensity of the reproductive event and depend on the searcher's ability to locate the species and the time invested searching (C. Pacheco, USFWS, pers. obs.). Although toad counts during reproductive events are considered a reliable method to estimate toad abundance, we cannot assume that all individuals are counted because not all toads (i.e., juveniles and non-reproductive sub adults) are detected during a reproductive event. Furthermore, the size of a wild PRCT population is difficult to determine due to lack of information regarding factors that may affect the form of population growth of the species. This uncertainty is exacerbated when the species has a large reproductive output followed by a low number of mature adults counted during next breeding events. Therefore, the lack of information on the population dynamics of the species is a limiting factor to determine what constitutes a viable population, and how to meet this criterion.

The recovery plan calls for five captive populations of 300 individuals each, for approximately 1,500 toads in captivity. Since 1982, AZA has maintained healthy individuals of PRCT in captivity demonstrating extensive knowledge in husbandry techniques on this species and producing significant number of tadpoles to be introduced in Puerto Rico. In 2013, the PRCT captive population consisted of about 602 toads (203 males, 155 females and 244 juveniles) in 31 AZA institutions in United State and Canada (Barber, 2013). With this amount of adult toads, AZA was able to produce in single year over 71,079 captive breed tadpoles and 520 toadlets to be released in five sites in Puerto Rico (Barber 2013). In 2014, the PRCT captive population has increased to 898 toads (324 males, 282 females and 292 juveniles) (Barber 2014). Presently, the captive population is comprised by toads from the southern population and crossing of northern and southern population. PRCTs from the northern population no longer exist in captivity. It is important to highlight that the number of captive toads and production of captive breed tadpoles may change year by year due to natural factors (i.e., natural mortality, toads age structure) and availability of resources (number of institutions involved that can hold toads, like zoos). In captivity, PRCTs are provided with appropriate environmental parameters, medical support and nutritional diet. Additionally, AZA has successfully maintained a studbook for the PRCT captive breeding program and

transfer plan of individuals among AZA institutions to ensure 95% genetic diversity is maintained for 100 years (Smith 2014). Currently, the knowledge on maintaining healthy PRCT captive population and captive breeding program has been well executed by AZA. However, AZA has recently expressed its concern about maintaining the amount of 1,500 toads in captivity to meet recovery criteria due to the lack of facilities and resources (Barber, AZA, 2014, pers. comm.). The challenge is exacerbated when two demes of the PRCT, the southern population and northern population, are managed separately in captivity. Therefore, we recommend that the criterion based on number of toads in captivity (i.e., 1,500 toads) should be reconsidered to better maintain both demes taking into consideration new information on the species and AZA expertise.

# C. Updated Information and Current Species Status

# 1. Biology and Habitat

# (a) Species' abundance, population trends (e.g. increasing, decreasing, stable), demographic features, or demographic trends

At the time the recovery plan was approved, information available on species abundance and population trends through its historical range was very limited to absent. The PRCT was believed to be extinct in Puerto Rico from 1932 to 1966, until it was rediscovered in the municipality of Isabela in 1966 (García-Díaz 1967), and later discovered in in the GCF in 1984 (Moreno and Canals 1985).

Since the rediscovery of the species, two populations of the PRCT have been recognized, one in the northern karst region and another in the southern karst region. For both populations, the PRCT population estimates have been derived from anecdotal reports, species expert opinion, number of tadpoles released and number of toads observed during reproductive events or single night surveys. Currently, toad counts during reproductive events have been considered a reliable method to estimate toad abundance in areas where the species occur naturally. As well, toad counts during night search, and number of tadpoles and toadlets released, provide some information about the status of the population in areas where the species is introduced. Aside from this information, no scientific data regarding the abundance of the species is available.

The current available information indicates that the number of PRCTs reported or documented has increased since the recovery plan was approved in 1992. By 1992, the abundance of the PRCT in Puerto Rico was estimated at around 2,000 individuals in one large population located in the southern karst region, and approximately 25 to 50 individuals in another population in the northern karst region (USFWS 1992). Johnson (1994) revised the original estimates to 300 toads for the southern population and no more than 25 toads for the northern population. However, Letini (2003) and G. Ross (former UPRM graduate student, 2005, unpubl. data) estimated again the abundance of the southern population at around 3,000 toads. Additionally, another natural reproductive population of PRCT was discovered in 2008 at Punta Ventana in the municipality of Guayanilla. Moreover, 312,000 tadpoles and 1,546 toadlets of PRCT have been released on seven re-introduction sites in Puerto Rico.

Unfortunately, the northern wild natural population is considered extirpated because no PRCTs have been detected since 1992 despite availability of suitable breeding habitat (Ross 2007; E. Estremera, Liga Ecológica de Quebradillas, 2014, pers. comm.).

Presently, the PRCT southern population is comprised by one large natural population and three reintroduction sites. The natural population occurs and breeds in three areas (i.e. GCF, Punta Ventana, and Ciénaga). These groups are located close enough (approximately 2.5 miles (4.0 km)) from each other and the interaction among PRCTs from different locations is likely to occur (Campbell 2014). Therefore, for the purpose of this review, we will refer to these three natural reproductive populations as sub-populations.

# **Natural Populations**

#### Guánica Commonwealth Forest

The PRCT was discovered in 1984 at the GCF by Moreno and Canals (1985), estimating the population at around 1,000 males and 5 females. Since 1984, the PRCT population at GCF has been estimated based on number of toads counted per breeding event (Table 1) (CBSG 2006; Canals, former GCF Manager, PRDNER, 2008, unpubl. data,). Information gathered by Canals reveals that number of toads per breeding events may fluctuated from two toads to 2,224 toads, and number of breeding events per year also fluctuated throughout the time. On October 9, 2005, G. Ross and M. Canals reported 2,224 toads (1,444 males and 780 females) in a single breeding event at the GCF (G. Ross, former UPRM graduate student, 2005, unpubl. data; M. Canals, GCF Manager, PRDNER, 2008, unpubl. data). From 1992 to 1998, no breeding events or toads were documented at the GCF (Joglar 2005). Otherwise, after 1998, 2 to 4 breeding events have been recorded several times in a single year (Table 1; M. Canals, GCF Manager, PRDNER, 2008, unpubl. data). Although Table 1 presents breeding events until 2008, at least four more PRCT breeding events are known to have occurred after 2008 in the GCF (C. Pacheco, USFWS, 2014, per. obs.). These breeding events are considered successful because thousands of recently metamorphosed toads were documented migrating into the forest. On September 8, 2013, after a rain precipitation of 2.54 inches, 35 PRCTs were documented in a breeding event. A Service biologist observed 22 PRCTs in amplexus (sexual embracement/mating position), 12 free swimming males and one female, and 10 egg mass (C. Pacheco, USFWS, 2013, per. obs.). Later, on November 3, 2013, a second breeding event was documented at the same site. This time, a Service biologist observed 11 toads (i.e., 10 males and 1 female) and 3 eggs mass. No amplexus was observed. These toads were found swimming free or heading back to the forest.

Toad counts during reproductive events are considered a reliable method to estimate toad abundance in the GCF. But, we cannot assume that all individuals are counted because not all toads (i.e., juveniles and non-reproductive sub adults) are detected during a reproduction event. Therefore, we believe the population size is underestimated and these numbers do not represent the entire population. Based on the information gathered during this review, we believe that the PRCT population in GCF should be considered stable because breeding events are occurring, and toads are frequently observed in the area.

Table 1. Dates and number of toads observed in breeding events at the Guánica Commonwealth Forest. (CBSG 2006, updated by M. Canals, GCF Manager, PRDNER, 2008, unpubl. data).

Date of breeding events	Number of PRCT		
July 3-5, 1984	800-1000*		
September 17-21, 1984	100*		
November 4-5, 1984	7		
October 5-6,1985	400-500*		
June 20-21, 1987	14		
November 26-27, 1987	100*		
August 24, 1988	64		
June 14, 1990	182		
October 18-21, 1990	8		
January 5, 1992	60*		
May 22, 1992	28		
September 21-22, 1998	216		
April 17-18, 2000	2		
August 22-23, 2000	6		
September 17-27, 2000	187		
May 7-10, 2001	35		
August 22, 2001	14		
April 20-22, 2002	40*		
September 13-15, 2002	42		
April 18-20, 2003	78		
October 8-9, 2003	143		
September 14-15, 2004	230		
November 10-13, 2004	6		
May 17-18, 2005	558		
July 5-7, 2005	130*		
July 19-20, 2005	43		
October 7-9, 2005	2224		
June 24-25, 2006	36		
August 24, 2007	132		
October 28, 2007	94		
August 16, 2008	8		
September 4, 2008	43		
September 22, 2008	368		

Number of toad estimated \*

#### Punta Ventana pond

The PRCT was first reported in the Punta Ventana area by José F. Sáez-Cintrón, who documented one adult male adjacent to a forested drainage that discharges its overflow into the Punta Ventana pond (J.F. Sáez, letter dated April 2, 2008; Barber 2009). Subsequently, after heavy rains in September 2008, Frank González reported at least three PRCT males calling for mates in an area close to a natural and ephemeral pond located in Punta Ventana (F.S. González, letter dated September 19, 2008, USFWS 2013a). On October 2, 2008, Service biologists and the land owner of the Punta Ventana property, conducted a visit to pond, and found 19 juvenile PRCTs (metamorphosed less than 15 mm (0.59 in) in snout to vent length (SVL)), and tadpoles (Powell and Guarnaccia 2013). Since 2008, the species has

been monitored in this site by the Service and by San Francisco Wind Farm (SFWF) biologists, gathering biological information on the species. Further observations provided evidence that this site harbors suitable feeding, sheltering and breeding habitat for the PRCT (USFWS 2013a).

Presently, the Punta Ventana pond is the only PRCT breeding site known in Bocas ward of Guayanilla (USFWS 2013a). Since the discovery of the species in this site, at least five breeding events have been known to occur in this pond. Unfortunately, these breeding events have not been monitored. Thus, the amount of reproductive females and males per breeding event is unknown. On March 28, 2012, during a heavy rain event, Service biologist Carlos Pacheco, and SFWF staff counted 132 males and 2 females within and surrounding the Punta Ventana breeding pond. Despite the relatively high number of males calling during that night, no toads in amplexus or egg masses were found the following morning. On September 10, 2013, C. Pacheco visited the Punta Ventana pond, the day after a heavy rain event, and observed 21 PRCT egg masses and numerous recently hatched tadpoles (less than 12 hour) swimming free, but no crested toads (C. Pacheco, USFWS, 2013, pers. obs.).

Recent PRCT surveys in Punta Ventana have only delivered number of toads per unit area or number of individuals per time search effort. The SFWF biologist has used relative abundance to estimate the species density at 2.1 toads per hectare (ha) within the SFWF project footprint (Powell and Guarnaccia 2013; USFWS 2013a). The SFWF biologist conducted toad counts in 1.8 ha of PRCT upland habitat within the total SFWF project footprint (i.e, 5.3 ha (13.1 ac)) and around the Punta Ventana breeding pond, as well as monitored toadlets dispersal after breeding events in 2011 and 2012 (Powell and Gurnaccia 2013). Maximum toad count for the survey period was on August 7, 2012, where A. Silva (SFWF biologist) counted 100 adults and sub-adults, including 48 males, 28 females, and 24 individuals of undetermined sex within the surveyed area. Otherwise, toadlets density varied depending on the timing of toadlet emergence and migration from the breeding pond and distance to the survey site. Maximum densities of 100-400 toadlets/m² were recorded along the Punta Ventana breeding pond after toadlets started to emerge on August 2012, and decreasing densities in later surveys (Powell and Gurnaccia 2013).

The PRCT appears to be most numerous along the cliff that surrounds the breeding pond in Punta Ventana, even when no breeding event is expected (Barber 2013; C. Pacheco, USFWS, 2014, per. obs.). On June 15, 2013, after a small rain event (i.e., less than 0.1 inches (2.5 mm) of rain), and the breeding pond still completely dry, three searchers found 34 PRCTs during a night search of 4 hours (i.e., 9 toads/hr) (Barber 2013). On November 6, 2013, under similar conditions, eleven searchers found 47 toads in 2 hours a night search (i.e., 23 toads/hr) around the pond (C. Pacheco, Service, unpubl. data, 2013). However, on June 13, 2014, during dry season (no rain events reported in more than 10 days), eight PRCTs were documented in 1.9 hours of night search (i.e., 4 toads/hr) (C. Pacheco, USFWS and D. Barber, AZA, 2014, unpubl. data.). This last finding is considered high because of the lack of rain.

Studies that provide more accurate estimates of population size at the Punta Ventana site are unavailable. However, based on the information gathered during this review, we believe that

the PRCT population in Punta Ventana should be considered as stable because breeding events are occurring and toads are observed in this site.

# Ciénaga wetland

Little is currently known about the species abundance in the Ciénaga wetland. In 1985, Moreno and Canals (DNER, 1985, unpubl. data) reported the Ciénaga wetland as an active PRCT breeding pond, documenting 20 toads at this wetland after heavy rainfall. However, by 1992, this population was thought to be extirpated because the species had not been observed there for several years (USFWS 1992). On May 2010, the PRCT was rediscovered at the Ciénaga wetland by USFWS biologists Carlos Pacheco and Jan Zegarra who documented one adult female and three adult males (USFWS, 2010, unpubl. data). No amplexus was observed. These toads were found swimming free or heading back to the forest. Aside from this anecdotic information, no scientific data regarding the abundance of the species in Ciénaga wetland is available.

# **Species Introduced Sites**

Releasing captive bred tadpoles into the wild is considered a powerful tool to enhance the recovery of the PRCT by increasing number of individuals in a particular area (USFWS 2013b). Since 1984, captive bred PRCTs have been released in seven locations in Puerto Rico; four sites in the northern karst region and three sites in the southern karst region. These release sites has been chosen to advance recovery goals for the species, and include populations in restored or stable habitat that the species have been extirpated. As well, all PRCT release sites are located within the historical range of the species. Therefore, for the purpose of this review, we will refer to these populations as re-introduced populations.

By 1989, about 800 captive breed toadlets were released in Cambalache Commonwealth Forest, but no more releases were conducted at this site due to lack suitability in the surrounded habitat (Paine 1985). However, efforts to re-introduce the PRCT are undergoing at Manglillo Grande in the GCF, at El Tallonal in Arecibo, at Gabia Farm in Coamo, at Río Encantado in Ciales/Florida, at La Esperanza in Manatí and Cueva El Convento in Guayanilla (Figure 3; Table 2).

Presently, the PRCT abundance at the introduction sites has not been quantified. The only information available is the number of tadpoles that have been released per site and the number of toads (adults and juveniles) that have been observed during night searches, and after tadpole metamorphosis. As of to date, over 312,000 tadpoles and 1,546 toadlets of PRCT have been released in Puerto Rico (Table 2; D. Barber, AZA, 2014, unpubl. data).

# Manglillo Grande

The introduction effort in Manglillo Grande started in 1992. To date, approximately 150,053 tadpoles and 1,026 toadlets have been released into this area (Table 2; D. Barber, AZA, 20214, unpubl. data). In 2003, M. Canals (GCF Manager, PRDNER, 2003, pers. comm.) observed 7 adult PRCTs in Manglillo Grande, two of them were in amplexus. Later in 2007,

numerous juveniles (more than 50 toads less than 1.5 cm (0.59 in) in length) were documented around the rearing pond before a scheduled tadpole release (C. Pacheco, USFWS, 2007, unpubl. data). Both events confirm that introduced captive breed tadpoles survived to adulthood and were able to reproduce. This is the first evidence that the tadpole release efforts have been successful (Barber 2007).

#### El Tallonal

At El Tallonal, PRCT tadpoles were first released in 2006. To date, over 55,887 tadpoles and 520 toadlets have been released in the area. Most of the PRCT tadpoles released here were offspring of pure northern lineage. As a result of these reintroduction efforts, adult toads are frequently observed around the breeding ponds. In 2007, two adult toads were documented on a trail close (about 100 meters) to the ponds (D. Barber, AZA, 2007, pers. comm.). That same year, members from the NGO *Iniciativa Herpetológica* (IH), Sondra Vega and Alberto Puente, found 5 adult toads (3 males and 2 females) in a single night (S. Vega, IH, 2007, pers. comm.). In 2008, El Tallonal property owner, Abel Vale, observed 18 adult males calling around the artificial pond during a heavy rain event (A. Vale, Ciudadanos del Karso (CDK), 2008, pers. comm.). Despite the number of males calling during that night, no toads in amplexus or egg masses were found the following morning (S. Vega, IH, 2008, pers. comm.). In 2014, six adult PRCTs were found during a 2.75 hours of night search (D. Barber, AZA, 2014, unpubl. data). Although adult females and males have been documented in the surrounding areas of the breeding ponds, no successful breeding event has been yet documented. Presently, PRCT population estimate at El Tallonal in Arecibo is not available. Nevertheless, because toads of different size classes (i.e., juveniles, sub adults and adults; female and male) are frequently sighted at this site despite no breeding events, we believe that the species is improving.

#### Gabia Farm

Gabia Farm is managed by PRDNER and is located less than one kilometer (0.62 miles) west to the Coamo Springs facilities. The Coamo Springs is the first PRCT collection site in the southern karst region (Grant 1932). No additional collections or sighting reports on PRCT at the Coamo Springs area had come to light since 1932, hence, this population is considered extirpated. The PRCT re-introduction efforts started at Gabia Farm in 2006. Since that time, approximately 42,597 captive bred tadpoles have been released in this site (Table 2; D. Barber, AZA, 2014, unpubl. data). In 2008, five adult male PRCTs were sighted calling around the breeding pond. Additionally, numerous toads (mean SVL = 25 mm (0.98 in)) have been observed in different sections of the property (J. Casanova, Gabia Farm Manager, PRDNER, 2008, pers. comm.). On May 13, 2010, eight toads (SVL = 2.2 cm to 6.5 cm) were found during a 1.4 hours night search (R. Cáceres, USFWS volunteer, 2010, unpubl. data). Although PRCTs are frequently sighted at this site, no breeding event has been documented yet.

#### Cueva El Convento

The re-introduction effort in Cueva El Convento started in 2012. To date, approximately 60,053 tadpoles have been released at this site in the past two years (Table 2; D. Barber, AZA, 2014, unpub. data). Since 2012, small PRCTs (SVL less than 25mm) are frequently sighted on the surrounding areas of the release pond. On June 19, 2014, Luz Morales-Sáez (Biologist, Para La Naturaleza (PLN), 2014, unpubl. data) reported a PRCT female (SVL= 67 mm) in the vicinity of the release pond. Based on our experience, the size of this toad corresponds to a two years old adult toad, most certainly from the 2012 release.

# Río Encantado and La Esperanza

The first release of PRCTs at Río Encantado was conducted on June 8, 2012, releasing 800 captive bred tadpoles. The second tadpole release was on June 14, 2014, releasing only 185 tadpoles. Despite the low number of tadpoles released at this site, PRCT has shown good survivorship. On June 6, 2014, Manuel Sepúlveda (PLN, 2014, unpubl. data) reported an adult PRCT male (SVL= 80 mm) in the vicinity of the release pond. Based on our experience, the size of this toad corresponded to a two years old adult toad from the 2012 release.

To date, only one PRCT tadpole release has been conducted in La Esperanza, Manatí. On July 31, 2013, 2,469 tadpoles were released at this site. Presently, PRCT juveniles are frequently sighted in the surrounding areas of the release pond (R. Rodríguez, PLN, 2014, pers. comm.).

Table 2. Amount of captive bred Puerto Rican crested toad (tadpoles and toadlets) released per re-introduction site in Puerto Rico (D. Barber, AZA, unpubl. data, 2014).

Re-introduction site	Amount of PRCT tadpoles released	Amount of PRCT toadlets released	Starting year	Current status of releases
Manglillo Grande	150,053*	1026*	1992	ongoing
El Tallonal	55,887*	520*	2006	ongoing
Gabia Farm	42,597*	-	2007	ongoing
Rio Encnatado	985*	-	2012	ongoing
Cueva el Convento	60,053*	-	2012	ongoing
La Esperanza	2,469*	-	2013	ongoing
Total:	312,044*	1,546*		

<sup>\*</sup>Total amount updated October 24, 2014

#### **Reproductive Biology**

Maturation time of the PRCT is known from the captive population, but little is known from the wild. In captivity, Paine (1985) observed maturation based on reproduction within 1 year of age, but he suggested that it probably does not occur before the second year of life in the wild (Service 1992). In the wild, M. Canals (PRDNER, 2005, unpubl. data) reported four significant reproductive events in a single year with approximately 10 to 780 adult females per reproductive event. During these reproductive events, M. Canals observed young males toads (approximately one year old) in amplexus, but only adult female toads (more than two years old) were sighted in the breeding ponds. After a year of re-introduction events, J.

Casanova (Gabia Farm Manager, PRDNER, 2009, pers. comm.) and A. Vale (CDK, 2009, pers. comm.), observed approximately one year old male toads calling around the breeding pond in Gabia and El Tallonal, respectively, but no females were sighted. Studies with other Bufonids and field observations suggest that the PRCT is a biannual breeder, probably because females must develop sufficient lipid storage to fuel egg development (USFWS 1992). However, no direct evidence from the PRCT in wild is available to support this argument.

Extremes in sex ratios have been reported for this species with low incidence of males in the northern population (Rivero et al. 1980), and low incidence of females in the southern population (Moreno 1985). However, new information on the species suggests that male PRCTs seem to be considerably more abundant than females in both linages. At the GCF, the sex ratio has been estimated at around 5 males per female (5:1) during a breeding event (M. Canals, former GCF Manager, PRDNER, 2008, unpubl. data). As well, information from PRCT surveys at El Tallonal show a sex ratio in favor to males (D. Barber, AZA, 2014, unpubl. data). The significance of these observations is difficult to assess without more information about the reproductive biology of this species.

# (b) Genetics, genetic variation, or trends in genetic variation (e.g. loss of genetic variation, genetic drift, inbreeding, etc)

When the recovery plan for the PRCT was approved, genetic research indicated that the two PRCT populations, northern and southern, were distinct and that must be managed separately (USFWS 1992). Preliminary data suggest that these populations have significant differences in mitochondrial DNA, reflecting their geographical separation (Johnson 1994; Goebel 1996). Recent work on genetic variation among species populations found only two moderate divergent mitochondrial haplotypes, with one fixed in each of the southern and northern lineages, and moderate genetic variation in microsatellite loci (Beauclerc et al. 2009). The northern population is moderately divergent from the southern populations at mtDNA, with 1.5% sequence divergence between the two haplotypes (Beauclerc et al. 2009). However such divergence between the two populations does not appear to warrant classification as separate subspecies (Beauclerc et al. 2009). Thus, Beauclerc et al. (2010) recommended that a third breeding colony be established in which northern and southern individual are combined because the northern breeding colony may not remain viable due to its small size and inbred nature.

The southern population, both captive and wild, has not suffered reduced genetic diversity, nor has it diverged substantially during its isolation (Beauclerc et al. 2009). However, the pure northern population no longer exists in captivity and the low number of individuals released in the wild is descended from four inbred siblings. It is probable that the pure northern colony will become extirpated due to demographic stochasticity or inbreeding depression (Beauclerc et al. 2009).

#### (c) Taxonomic classification or changes in nomenclature.

The PRCT was originally described as *Peltoprhyne lemur* in 1868 by E.D. Cope (Cope 1868; Stejnerger 1902). In 1902, Stejneger (1902) placed the PRCT into the genus *Bufo* (Service 1992). However, Pregill (1981) found that the species was misplaced under the genus *Bufo* and recognized the name *Peltophryne*.

Presently, morphological and molecular data support the monophyly of some toads endemic to the West Indies that had been placed between the genus *Peltophryne* and *Bufo*, including the PRCT (Schwartz and Henderson 1991; Pramuk 2000; Pramuk et al 2001). However, some studies on immunological distance (Hedges 1996; Pramuk et al 2001) and mitochondrial DNA sequences (Graybeal 1997) suggested that the genus Peltophryne should be nested within a paraphyletic Bufo. As such, Hedges (1996) and Pramuk et al. (2001) recommend the name Peltophryne should be again synonymize with Bufo. Pramuk et al. (2001) also suggests that the West Indian toads should be grouped into a New World origin group under the Bufo peltocephala Group. Meanwhile, Frost et al. (2006) proposed that the large genus Bufo should be partitioned into a number of distinct genera, including *Peltophryne*. Results of the most recent study on global bufonid history suggest that the native bufonids of the Greater Antilles (Cuba, Hispanola, and Puerto Rico) should be kept under the genus *Peltophryne* due to their presumed monophyletic origin (Alonso et al. 2012). Currently, the Integrated Taxonomic Information System (ITIS) recognized the genera Peltophryne as valid for the West Indian Toads (http://www.itis.gov; accessed on march 17, 2014; last updated 03-mar-2014). As this is the most recent treatment of West Indian toad taxonomy, we will refer to the Puerto Rican crested toad as *Peltophryne lemur*.

(d) Spatial distribution, trends in spatial distribution (e.g. increasingly fragmented, increased numbers of corridors, etc.), or historic range (e.g. corrections to the historical range, change in distribution of the species within its historic range, etc)

The PRCT has been described as a Caribbean endemic toad with occurrences known only from Puerto Rico and Virgin Gorda in the British Virgin Islands (BVI) (52 FR 28828; USFWS 1992).

At the time the recovery plan was approved, the PRCT distribution in Puerto Rico was limited to two isolated populations, one in the municipality of Quebradillas in the northern karst region, and another in the GCF in the southern karst region. These both 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 (USFWS 1992). At that time, the species was also known to occur historically from six municipalities, one re-introduction site at the Cambalache Commonwealth Forest, and one location in Virgin Gorda (Figure 1; USFWS 1992). The fate of the PRCTs released at the Cambalache Commonwealth Forest is unknown because the species has not been monitored at this site. As well, the PRCT was believed to be extinct in from Virgin Gorda because it has not been reported in many years on that island (USFWS 1992).

New information gathered during this review suggests that the PRCT had wider range in the Caribbean and distribution in Puerto Rico than we previously thought in 1992 (Figure 2). Many of the locations where the species occurred have been derived from anecdotal reports

and not from scientific studies. Therefore, the information about the species collection sites and sightings is limited and not accurate. Some anecdotal reports have been verified, others need verification. Additionally, the PRCT distribution in Puerto Rico has increased since the species was listed in 1987 due to the implementation of recovery actions (Figure 3). Since 1992, efforts have been concentrated on searching for the species in its historical collection sites and other suitable areas in Puerto Rico, and on re-introducing the species throughout its historical range (CBSG 2006).

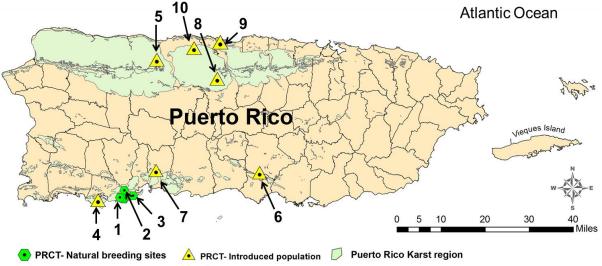
In Puerto Rico, the PRCT has been documented to historically occur in 14 scattered sites along the northern and southern karst regions: eight in the northern karst, and six in the southern karst (Figure 2). The species was collected in the municipalities of Bayamón, Vega Baja, Barceloneta, Arecibo, Quebradillas, Isabela, Coamo, Santa Isabel, Ponce, Guayanilla, Yauco and Guánica (Grant 1932; Service 1992; Rivero 1998). Fossil records are known from Camuy and Morovis (Pregill and Olson 1981; Rivero 1998, Goebel 1996). Additionally, the species was anecdotally reported in the municipalities of Peñuelas, San Germán, Aguadilla, Manatí, Dorado and Vieques Island (Stejneger 1902; Rivero 1998; J. Ortíz, UPRM, perns. comm 2009). L. Stejneger (1902) and Barbour (1917) mentioned that the PRCT had been sighted in Vieques Island, but he failed to obtain a specimen.

Anecdotic reports suggest that the PRCT used to occur on other Caribbean Islands such as St. Thomas and St. John (U.S. Virgin Islands). Barbour (1917) mentioned that the PRCT occasionally appeared in St. Thomas carried on lumber from Haití and Vieques Island. Robert L. Norton, professor at the Santa Fe Community College in Florida, reported a sighting of a PRCT while he was surveying wildlife in Lameshur Bay in St. John, USVI (Norton 1997). R. L. Norton did not collect a specimen at that time due to the restriction for collection of wildlife within a National Park. These sightings were well documented by Barbour (1917) and Norton (1997), providing some detailed information regarding the species description and the site where the crested toads were found. However, the PRCT occurrence in USVI is considered anecdotic since it has not been collected there. These anecdotal reports should be considered as new information for this review, expanding the PRCT historical range to the islands of St. Thomas and St. John.



Figure 2. Map of the Puerto Rican Shelf indicating the localities where the Puerto Rican crested toad (*Peltophryne lemur*) has been documented (collected or sighted) (USFWS, 2014, unpubl. data).

Now the PRCT is thought to be found exclusively in Puerto Rico since it has never been collected in USVI, and is considered extirpated from Virgin Gorda. Currently, the PRCT is known to occur in three natural populations and six reintroduced sites (Figure 3).



(1) Tamarindo, Aroma and Atolladora – Guánica Commonwealth Forest; (2) Cienaga - Yauco; (3) Punta Ventana - Guayanilla; (4) Manglillo Grande – Guánica Commonwealth Forest; (5) El Tallonal - Arecibo; (6) Gabias' Farm - Coamo; (7) El Convento Natural Reserve - Guayanilla; (8) Rio Encantado Natural Reserve – Ciales/Florida; (9) Hacienda La Esperanza Natural Reserve – Manatí; (10) Cambalache Commonwealth Forest – Arecibo (status: unknown)

Figure 3. Current distribution of the Puerto Rican crested toad (*Peltophryne lemur*) in Puerto Rico (USFWS, 2014, unpubl. data).

The PRCT naturally occur in a large population comprised by three reproductive sub populations located among the municipalities of Guánica, Yauco, and Guayanilla in southern Puerto Rico (Figure 4). In these municipalities, most of the sightings occur within the GCF, Punta Ventana pond area, and Ciénaga weltland, all located approximately 2.5 miles from each other (4.0 km) (Figure 4).

The GCF is a public land managed by the PRDNER for conservation since 1976 (DNER 1976). This forest falls within the municipalities of Guánica, Yauco and Guayanilla. The species was reported in three breeding ponds within this forest: Atolladora, Aroma, and Tamarindo (Figure 4); being Atolladora the site where the species was first discovered, and Tamarindo the primary breeding pond (M. Canals, former GCF Manager, PRDNER, 2008, unpubl. data). These are considered the main breeding ponds for the species (Johnson 1990), although other possible natural and artificial breeding ponds may occur within the boundaries of the GCF. For the purpose of this review, these three breeding sites will be referenced as one PRCT population in the GCF because of the proximity among the three ponds (i.e., less than 500 linear meters / 1,640 feet). Since 1984, this population has been well monitored and it is considered as stable. Most of the PRCT reports within the GCF are associated to reproductive events and sightings in the surrounding areas to the breeding ponds. However, some sightings of the PRTC suggest the species may be more widely distributed inside the forest. M. Canals (former GCF Manager, PRDNER, 2006, pers. comm) observed several small toads (less than 3 cm) at the Eugenia's gorge. This gorge is located at approximately 0.6 miles (1.03 km) north from the Tamarindo breeding pond. On August 2008, USFWS biologist C. Pacheco (USFWS, 2008, unpubl. data) found an adult male PRCT dead at km 8.7 of State road PR 333, approximately 33 feet (10 m) east from the gate of the Ballenas trails. Ballenas trail is located approximately 1.2 mile (1.9 km) west from Atolladora pond. In April 2008, Mr. José F. Sáez-Cintrón (member of community group *Coalición Bosque Seco*) reported an adult toad on the eastern boundary of the GCF, 0.8 miles (1.3 km) from the Punta Ventana breeding pond (J.F. Sáez-Cintrón letter to USFWS dated April 2, 2008). In 1987 and 1989, PRCT toadlets were released at *Hoya Honda* and *Ojo de Agua*, both sites are located at approximately 2.3 mile (3.7 km) northwest of the Tamarindo natural breeding pond in the GCF (Figure 4). Presently, the success of these releases at the GCF is uncertain because the species has been poorly monitored in those areas.

The Ciénaga wetland is on privately-owned land located at Barinas Ward in the municipality of Yauco, approximately 2.0 miles (3.2 km) north of the GCF breeding ponds (Figure 4). The PRCT breeding pond in Ciénaga was first discovered in 1985 (Moreno and Canals, DNR, 1985, unpubl. Data), and later re-discovered by Service biologists in 2010, (Pacheco, USFWS, 2010, unpubl. data) after many years believed extirpated. At this site, the breeding pond is located on a floodplain of an intermittent stream that drains a large area of the GCF (Moreno and Canals, DNR, 1985, unpubl. data). Unfortunately, the PRCT has been poorly monitored in this site; therefore, its status at Ciénaga is uncertain.

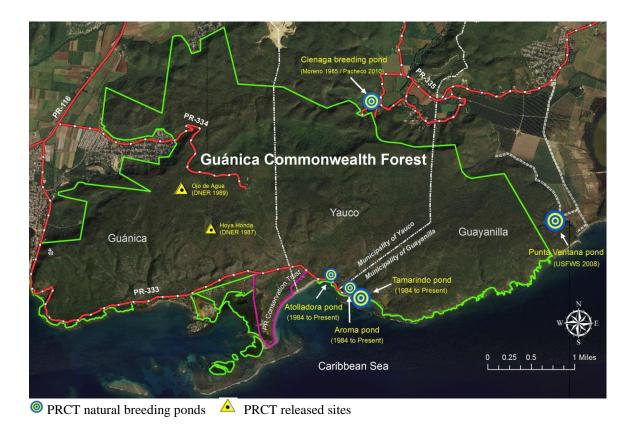


Figure 4. Map showing the Puerto Rican crested toad breeding ponds and release sites within and around the Guánica Commonwealth Forest. (USFWS, 2014, unpubl. data)

In 2008, another PRCT natural breeding pond was discovered in a privately-owned land located at Punta Ventana in the municipality of Guayanilla (Figure 4). The PRCT was first sighted here on September 2008 by Mr. Frank González (member of community group *Coalición Bosque Seco*), who reported at least three male PRCTs calling from a natural and ephemeral pond located approximately 2.5 miles (4.0 km) northeast of the GCF breeding pond (F.S. González latter to USFWS dated September 19, 2008, USFWS 2013a), and at approximately 2.6 miles (4.2 km) southeast from the Ciénaga wetland (Figure 4). On October 2, 2008, USFWS biologists and Mr. Victor González, owner of the property, conducted a site visit to the Punta Ventana pond and found a natural and reproductive population of PRCT (Powell and Guarnaccia 2013). Hundreds of tadpoles and several recently metamorphosed PRCTs were observed at the edges of the natural pond. Further site visits have confirmed the continued occurrence of the species at this site.

Efforts to locate the PRCT in the northern karst region have been conducted since 1992, but the species has not been found (Barber 2007b). In 2003, Gail Ross, a graduate student from the University of Puerto Rico, Mayagüez Campus, searched for the species at the historical sites in Quebradillas, and Isabela by using sound recorders, but detected no PRCTs (Ross 2007).

Since 1992, the USFWS, PRDNER, and AZA have concentrated efforts on re-introducing the PRCT in six sites within the species historical range in Puerto Rico (Figure 3). Currently,

PRCTs are being released at Manglillo Grande in Guánica, El Tallonal in Arecibo, Gabia Farm in Coamo, El Convento in Guayanilla, La Esperanza in Manatí and Río Encantado, a reintroduction site located in the boundary of the municipalities of Ciales and Florida.

By 1992, PRDNER constructed a concrete pond at Manglillo Grande to release captive-bred PRCT tadpoles. The Manglillo Grande pond is located 6.7 miles (10.8 km) west of the natural population in the GFC, but geographically separated by the Guánica Bay. This reintroduction site falls in the western section of the GCF and is located 0.22 miles (0.35 km) east of the Playa Santa community. Presently, the species is considered successfully introduced at Manglillo Grande since adult toads, reproductive events, tadpoles and juveniles have been observed in the area.

In 2005, the USFWS, through the Endangered Species and Partner for Fish and Wildlife Programs, provided funds and technical assistant to two locals NGOs: *Iniciativa Herpetológica* (HI) and *Ciudadanos del Karso* (CDK), for the construction of three artificial ponds and the re-introduction of the PRCT in the northern karst of Puerto Rico. The ponds were constructed at El Tallonal in Arecibo, a private land managed by CDK for conservation. El Tallonal is located 13 miles (20.9 km) southeast of the PRCT population that once existed in the municipality of Quebradillas. Presently, adult PRCTs have been observed after some rain events (A. Vale, CDK, 2008, pers. comm.; A. Puente-Rolón, IH, 2014, pers comm.).

The Gabia Farm is located in the boundary of San Idelfonso and Los Llanos Wards in the municipality of Coamo, and falls within the historical collection area of the species in southern Puerto Rico (Figure 5). The PRCT was first collected on the southern karst region in 1919 by Dr. K. P. Schmidt (C.A.S. Spec # 54989) at the Coamo Springs area in the municipality of Coamo (Grant 1932; Goebel 1996). Subsequently, the species was collected at the same area by S. Danforth in 1929 (UPRM Spec. # 1482-1484) and C. Grant in 1931 (Michigan Spec #73523); being Grant its last collector at the Coamo Springs area (Grant 1932; Goebel 1996). It is important to highlight that C. Grant found one crested toad 1 mile north of the Coamo Springs (Figure 5). Since 1932 no additional collections or sightings of PRCTs at the Coamo Springs area have come to light, hence, this population is considered extirpated. However, in 1976, Dr. Richard Thomas (Professor, UPR-Río Piedras) found one male PRCT in Río Descalabrado Ward, municipality of Santa Isabel. The collection site is located 2.28 miles (3.66 km) southwest from the Coamo Spring site (Figure 5). Since 1976, efforts have been conducted to locate the PRCT at the Río Descalabrado and Coamo Springs areas without success (R. Thomas, Professor, UPR-Río Piedras, 2005, pers comm.). In 2005, PRDNER nominated Gabia Farm as a re-introduction site for the species (Canals and Casanova, PRDNER, 2008, unpubl. data). Subsequently, in 2006, PRDNER staff constructed a concrete pond to release PRCT tadpoles at Gabia Farm, and later in 2009, they constructed another bigger pond to support PRCT breeding events. Both ponds were constructed with funding provided by the USFWS Coastal and Endangered Species Programs. Since 2007, seven PRCT releases have been conducted at this site and PRCTs have been sighted afterwards.

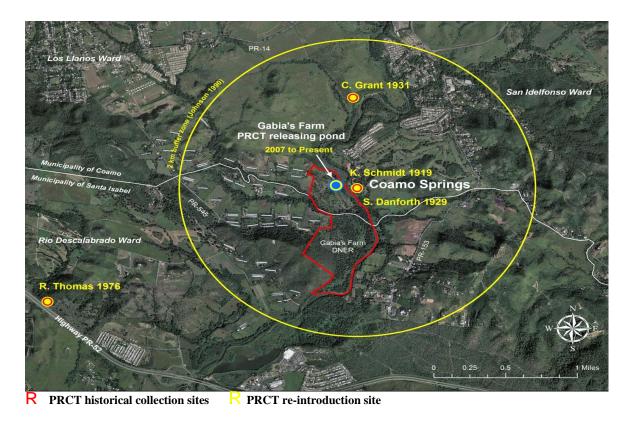


Figure 5. Historic and current distribution of PRCT at the Gabia Sector in Coamo, Puerto Rico (USFWS, 2014, unpubl. data)

In 2009, the USFWS and PLN signed two Cooperative Agreements to introduce PRCTs in three properties managed by PLN for conservation: two in the northern karst, and another in the southern karst. Cooperative Agreement F09AC00204 provided funds through the USFWS Endangered Species and Partners for Wildlife Programs for the construction of three artificial ponds: two at Río Encantado Natural Protected Area, and another at Hacienda La Esperanza Natural Reserve. These two protected areas fall within the historical range of the species in the northern karst region. In addition, Cooperative Agreement F09AC00209 provided funds through the USFWS Endangered Species and Coastal Program Programs for the construction of an artificial pond at Cueva El Convento. This site is located within the historical range of the species in the southern karst region of Puerto Rico. The natural features presented in these sites (e.g., rain fall, topography, soil, and forest structure) provide suitable habitat and is contributing to the successful establishment of a PRCT population, which is evidenced by the frequent sighting of individuals after initial releases.

The Río Encantado Natural Protected Area consists of several parcels of land located between the municipalities of Florida and Ciales managed by PLN for conservation. This reintroduction site is located at approximately 26.0 miles (42.2 km) east from the natural population site in Quebradillas, and about 13.2 miles (21.3 km) east from El Tallonal in Arecibo, at an elevation of 650 feet (198.1 m) from sea level (Figure 3). The first PRCT tadpoles release in this site occurred in 2012 (Table 2).

Hacienda La Esperanza Natural Reserve is located in the municipality of Manatí. The reintroduction site here is located approximately 7.6 miles (12.2 km) north of the Río Encantado Natural Protected Area, and 15.0 miles (24.1 km) east from El Tallonal in Arecibo, at elevation of 50 feet (15.2 m) of sea level (Figure 3). The first PRCT tadpoles release in this site occurred in 2013 (Table 2).

Cueva El Convento is located in the municipality of Guayanilla. This re-introduction site is located at 6.8 miles (10.9 km) northeast from the Punta Ventana natural population, and 24.8 miles (39.8 km) west from the historical collection site in Coamo Springs. The first PRCT tadpoles release in this site occurred in 2012 (Table 2).

Based on the information gathered for this review, the PRCT historical range and spatial distribution of the species in Puerto Rico has expanded since the species was listed. It is believed that additional PRCT populations might exist in the northern karst region and southern karst region because of the amount of suitable habitat available and recent sighting reports. However, the PRCT apparently stills restricted to Puerto Rico since as it has never been collected in USVI, and is considered extirpated from Virgin Gorda, BVI.

# (e) New information addressing habitat or ecosystem condition (e.g. amount, distribution, and suitability of the habitat or ecosystem)

At the time of listing, the PRCT was believed to occur only in subtropical dry forest. Back then, the species' habitat was described as areas of low elevation (not exceeding 650 feet (200 m) from sea level), on arid or semiarid, rocky areas with abundance of limestone fissures and cavities in well-drained soils (52 FR 28829). The species was also reported in a grassy field in Arecibo (USFWS 1992). Currently, the PRCT occurs in two forest associations: subtropical dry forest in the southern karst region (USFWS 1992; Norton 1998), and subtropical moist forest in the northern karst region (Pacheco, USFWS, 2014, pers. obs). The subtropical dry forest zone covers approximately 14% (317,332.73 acres (128,420 ha; 1,284.2 km<sup>2</sup>.)) of Puerto Rico and USVI (Ewel and Whitmore 1973). The dry forest habitat is characterized by small (<15 feet/5m) deciduous trees with small, coriaceous or succulent leaves and thorns, spines, and with a rainfall less than 30 inches (750mm) per years (Ewel and Whitmore 1973). The subtropical moist forest in Puerto Rico covers approximately 60.5% (1,316,107.9 acres; (532,610 ha; 5,326.1 km<sup>2</sup>) of Puerto Rico (Ewel and Whitmore 1973). This life zone is characterized by low variability in annual temperature and high levels of rainfall (>43.0 inches/1100 mm annually), forest composition dominated by semi-evergreen and evergreen deciduous tree species, and sizes up to 60 feet (20 m) tall, with rounded crown. Based on this information, possible habitat for the PRCT is widely distributed throughout Puerto Rico. Therefore, the amount of unused available suitable habitat suggests that the species may have more specific requirements or factors limiting its range. Hence, further studies would be needed to determine the species' limited distribution.

At the GCF, the PRCT has been found in the upland deciduous forest, semi-evergreen forest and scrub forest. The upland deciduous forest occupies 65% (approximately 6,000 acres; 2,428.11 ha.)) of the GCF area. The upland deciduous forest is mostly composed of trees, bushes and succulent plants with abundant leaf litter. The average temperature of this type of

habitat is 27.87°C (82.16°F) with average relative humidity of 76.32% (Matos-Torres 2006). The semi-evergreen forest occupies 19.8% (approximately 1,792 acres; 725.19 ha.) of the GCF area. This type of forest is associated to natural drainages, and is mainly composed of evergreen arborous species with a leaf litter covered surface. The average temperature at this type of forest is 27.09°C (80.76°F) with an average relative humidity of 83.11% (Matos-Torres 2006). Matos-Torres (2006) observed male toads on the limestone scrub or cactus scrub around the Tamarindo pond. Limestone scrub is a type of vegetation mostly composed of xerophytic arboreus species and bare rock surface. In this type of habitat the average temperature is 27.72°C (81.89°F) with an average relative humidity of 66.63% (Matos-Torres 2006). No permanent fresh water bodies are expected to be found in Limestone scrub (Lentini 2003).

The amount of protected habitat for the PRCT in the GCF has increased in the last 12 years. In 2003, the PRDNER acquired approximately 200 acres (80.93 ha.) of dry forest to the north of the GCF (M. Canals, former GCF Manager, PRDNER, 2008, pers. comm.). These lands harbor suitable habitat for the species and are closed to the breeding site of Ciénaga wetland. Additionally, SFWF is proposing to transfer a total of 165.6 acres (67 ha) of dry forest habitat in Punta Ventana to the PRDNER as part of the mitigation for the construction of a wind energy farm (Powell and Guarnaccia 2013). The proposed land is contiguous to the GCF and harbors suitable habitat for the species (i.e., feeding, shelter, and breeding habitat) (USFWS 2013a). This parcel will be added to the 416 acres (168.3 ha) of dry forest habitat already transferred in the Punta Verraco and Cerro Toro in Guayanilla (Powell and Guarnaccia 2013). The addition of these lands to a Commonwealth forest may enhance the conservation status of the PRCT in the southern karst region because of their potential for the establishment of additional populations. Range expansion of the PRCT can occur if new breeding sites are selected (Johnson 1994). Some ponds located in these lands may be secondary breeding sites, when preferred sites are unavailable due to annual weather changes or habitat alteration.

The PRCT appears to prefer arid or semi-arid, rocky areas with abundance of limestone fissures and cavities in well-drained soil (Rivero et al. 1980; Moreno 1985; Paine 1985; Service 1992). However, Moreno (1985) mentioned that the species was collected on a grassy field in Arecibo. On May 16, 2012, USFWS biologists Carlos Pacheco and José Cruz found seven toads in small holes on the ground close to the watering system and on under the leaf litter beneath the trees of a mango tree plantation managed by Tropical Fruit, Inc. in Guayanilla (Pacheco, USFWS, 2012, unpubl. data). This site is located .75 miles (1.12 km) north of the Punta Ventana breeding pond.

Since 2003, the Service, PRDNER, and the local NGO CDK, have promoted the conservation and protection of the suitable habitat for the PRCT in State and privately-owned land in the northern karst of Puerto Rico. The Service is promoting the improvement and conservation of PRCT habitat on privately-owned land through the implementation of restoration practices under the Partners for Fish and Wildlife Program, Coastal Program, and the Endangered Species Program, and Section 6, Section 7, and Section 10 of the ESA. Currently, five private land owners around El Tallonal are improving habitat for the species through conservation agreements with the Service (Figure 6). In addition, CDK and PLN are acquiring more lands with suitable habitat for the PRCT in the northern karst region of Puerto Rico.

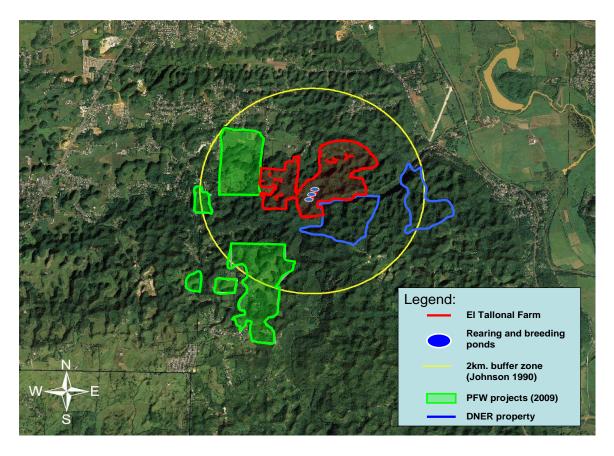


Figure 6. Distribution of private lands under agreements with the USFWS for the conservation and improvement of PRCT habitat around El Tallonal (USFWS, 2009, unpubl. data).

# (f) Other relevant information

Species Behavior:

Very little natural history information has been documented for the PRCT due to its rarity and semi-fossorial nature. Presently, known information on the species is regarding its mating behavior, post-reproductive movements, and defensive behavior.

The mating behavior and the post-reproductive movement of the PRCT in the wild have been studied since 1985 (USFWS 1992). It has been found that PRCT breeding events are triggered by heavy rains (i.e., more than 3 inches of rain within a 24 hour period) and the formation of temporary ponds in which adult males gather and start calling to attract females (USFWS 1992). Additionally, species experts suggests that drop in barometric pressure during atmospheric disturbances (i.e., tropical storms or hurricanes) may trigger the movement of reproductive toads toward breeding sites (M. Canals, former GCF Manager, PRDNER, 2008, unpubl. data). This weather is most likely to occur during the rainy season, which is normally from April to May, and during the peak of the hurricane season from August to October, being hurricanes the primary trigger for reproduction. Hurricanes do not occur every year, therefore, breeding events are sometimes infrequent (Ross 2005).

Johnson (1990) studied the post reproductive movement of the species at the GCF, following 11 radio tracked toads during three weeks. The radio tracked toads moved up to 60 meters (196.85 ft) the first night after release, and migrated up to two kilometers in 20 days (Johnson 1994). After a three-days period of activity in which the distance traveled averaged 150 meters (492.13 ft) during a 24-hour period (daily individual movement ranged from 15 to 500 meters (49.21 to 1,640.42 ft), toads settled into an area, and movement was reduced to an average of six meters (19.68 ft) per night. During the period of reduced movement, toads often returned to the same refuge.

Outside of breeding events, the species is difficult to detect because when disturbed, it often remains still and relies on its cryptic camouflage to avoid detection (Schmidt 1928; C. Pacheco, USFWS, pers. obs.). PRCTs have been observed using small holes and crevices to access the underground chambers as daytime retreats. These animals are able to scramble up vertical rock faces and steep dirt banks to find holes and crevices located at 30.5 cm to 2.13 m (12 in to 7 ft) above ground for refugia during daytime (Lentini 2003; Pacheco and Barber 2013). At Punta Ventana, PRCTs have been documented in holes and crevices located at vertical distance ranging from 30.48 cm to 1.52 m (12 in to 5 ft) of the ground. However, at El Tallonal, PRCTs are frequently found on a steep vertical dirt bank of 2.13 meters (7 ft) above the ground. These holes are mostly excavated and abandoned by Todus mexicanus, an endemic bird species that utilizes hillsides to excavate its nests (C. Pacheco, USFWS, pers. obs; Pacheco and Barber 2013). In forested areas and grass lands where the topography has low relief (i.e., rocks, slopes), and limited structural complexity (holes and crevices), adult toads have been found inside dead logs (J. Casanova, Gabia Farm Manager, PRDNER, 2008, pers. comm.), and inside empty nests of tarantulas (Cyrtopholis portoricae) (Pacheco and Barber 2013). Toads have been observed utilizing the same holes as permanent refuge.

Tadpoles metamorphosis and toadlets emergence from the ponds usually start to occur at 2-3 weeks (or 14 to 21 days) of development in the pond, but may last more depending on water temperature, tadpole growth and other factors (USFWS 2013a, Cáceres 2014). Once tadpoles complete metamorphosis and emerge from the ponds, toadlets will begin migrating into adjacent forest and uplands. Toadlets dispersion from the breeding site is unlikely to be random, yet not all toadlets migrate in same direction at the same time. M. Canals and J. Casanova (PRDNER, 2008, unpubl. data) monitored newly metamorphosed toads in Gabia as they left the rearing pond using fluorescent powder. They observed that toads sought refuge as soon as they left the water. Clumping behavior was also observed in the PRCT, typically 15-30 toadlets clump or group in one mass under any available object, conforming to the shape of the space for shelter. Toads of about 0.78 inches (2 cm) in length migrated up to 62 feet (19 m) in one night (M. Canals and J. Casanova, PRDNER, 2008, unpubl. data). One toadlet of 1 inch (1.5 cm) traveled 46 feet (14 m) in seven hours. On November 2, 2005, newly metamorphosed toads were observed climbing a steep limestone rock of 3.9 feet (1.2 m) above ground in search of holes for refuge in GCF (Pacheco and Barber 2013). In upland areas, toadlets seem to be more active and visible during daylight hours (A. Silva, SFWF, 2012, pers. comm.; Powell and Guarnaccia 2013).

PRCTs are often found on, under, or in dead-end holes and crevices in limestone bedrocks and on the ground where they appear to seek protection from predators and refuge from the heat during day light (Schmidt 1928; Rivero et al. 1980; Johnson 1990; Pacheco and Barber 2013). Toads have been documented using holes ranging from 0.6 to 1.8 inches (1.5 to 4.5 cm) wide and 0.8 to 1.8 inches (2.0 to 4.5 cm) high (Pacheco and Barber 2013). All toads inside holes were found sitting with their heads facing outward from the side of large boulders. Depths of the holes from the rock surface to the head of each toad varied from 1.3 to 3.5 inches (3.5 to 9.0 cm). Once approached, toads pulled backward, deeper into their holes, and tucked their lower jaw to the chest so that the bony top of the head formed a shield blocking the rest of the body. Several toads were observed pulling their closed eyes deep into their socket for additional protection (Pacheco and Barber 2013).

Abiotic factors that may affect growth and survival of the PRCTs tadpoles:

Abiotic factors such as salinity, temperature, and water volume may affect PRCT growth and survival during earliest development stages. Salinity has raised concern on species experts due to an increase in salinization of some natural breeding ponds and to their location close to the coast and sea level rise (M. Canals, former GCF Manager, PRDNER, 2008, unpubl. data; Cáceres and Ortiz 2014). Recent observations conducted by R. Cáceres (Center for Applied Tropical Ecology Conservation; CATEC, UPR-RP) revealed that water salinity concentrations higher than 8 parts per thousand (ppt) in the breeding ponds killed PRCT eggs and tadpoles, which may affect the survival and development of the species. Tadpoles at 8 ppt may survive, but take more time to metamorphose (up to 70 days; Cáceres, CATEC, 2014, unpubl. data). Salinity also affects their development as tadpoles at 8 ppt were smaller than those tadpoles raised at lower salinity concentration (Cáceres, CATEC, 2014, unpubl. data). Additionally, temperature and water volume may affect the development and time for metamorphosis of PRCT tadpoles. Tadpoles reared at 26°C and constant water level attained the highest body weight (i.e., 0.22g), and took an average of 27 days to metamorphose. However, tadpoles reared at 35°C and decreasing water levels presented lesser body weight (i.e., 12g) and metamorphosed a week earlier (Cáceres, CATEC, 2014, unpubl. data). Average time for PRCT metamorphosis in captivity ranged from 33 to 35 days (Lentini 2007).

Effects of the presence of the exotic giant marine toad (Rhinella marina) in PRCT breeding ponds:

It has been speculated that the presence of the marine toad (*Rhinella marina*; former *Bufo marinus*) in the breeding ponds has been detrimental to the PRCT (Ross 2005; Cáceres 2014). Marine toads are often documented using the same ponds that PRCT uses for reproduction. Schmidt (1928), and Paine and Duval (1985) suggested that marine toads compete with the crested toad for spawning sites, food and habitat; affecting the survivorship of early development stages (i.e., tadpoles and toadlets) of the PRCT. Though, Rivero et al. (1980) believed that the PRCT was scarce before the introduction of the marine toad, they did not discarded the possibility of competition between the two species. Indeed, marine toad tadpoles are known to prey on younger cohorts of their own species (Alford et al. 1995), although not heavily on other species (Crossland 1998; Crossland et al. 2011). In fact, Flores-

Vallejo (2011) documented a marine toad eating a recent metamorphosed PRCT at El Tallonal.

The high densities and the ability of marine toads to lay more eggs than PRCTs may add a competitive stress to the survival during early stages. Johnson and Paine (1990) observed the metamorphosis and dispersal of both the marine toad and PRCT from the Tamarindo breeding pond in GCF. Despite the removal of a number of marine toads by PRDNER staff, the estimated number of individuals per species still was being in favor of the marine toads. In 1998, GCF staff started again removing marine toads from the Tamarindo pond. In October 2005, G. Ross monitored the metamorphosis and dispersal of both species at the Tamarindo breeding pond and estimated that the number of individuals per species was in favor of the PRCT. A total of 6,852 PRCT toadlets and 4,524 marine toadlets were counted at 30 randomly selected sites over eight days survey (Ross, former USFWS volunteer, 2005, unpubl. data). Peaks of both species were observed on day 24 of development (Figure 7).

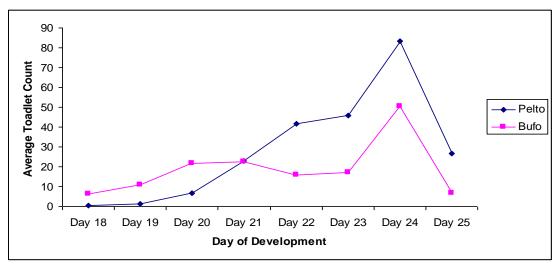


Figure 7. Average number of toadlets of Puerto Rican crested toads versus marine toads emerging per day in the Tamarindo breeding pond, Guánica Commonwealth Forest. Data collected from day 18 (October 29<sup>th</sup>) through day 25<sup>th</sup> (November 5<sup>th</sup>) of PRCT development (G. Ross, former USFWS volunteer, 2005, unpubl. data).

# Captive breeding program:

It is known that maintaining healthy captive population of PRCTs ensure the success of the captive breeding program and eventuality the reintroduction success (Miller 1985; Johnson 1990; USFWS 1992; Lentini 2003; Barber 2008). The PRCT captive breeding program started in 1980. At that time, Dr. Juan A Rivero (Professor University of Puerto Rico, Mayagüez) collected PRCTs from the northern population (2 females:2 males) and kept in captivity at the Puerto Rico Zoo (USFWS 1992). Progeny from these toads were placed at the Buffalo Zoological Garden and the Brookfield Zoo for reproduction (USFWS 1992). In 1982, Rick Paine, species coordinator of the PRCT in the SSP, captured three males of crested toads from the wild, whose offspring were destined for the SSP breeding program at Buffalo Zoo (Paine 1983). Later, in 1985, 20 toadlets were collected from the GCF (Johnson 1990,

USFWS 1992). In December 2007, 20 newly metamorphosed toads from GCF were collected and taken to the Fort Worth Zoo to serve as potential new founders and augment the captive southern population (Barber 2007). Subsequently, these PRCTs formed the basis for the captive breeding population managed under the AZA-SSP program.

Since 1982, AZA has maintained healthy individuals of PRCT in captivity (USFWS 2013b). AZA has been following the guidance published in *The Husbandry Manual for the Puerto Rican Crested Toad* (Lentini 2007), which describes protocols on specific areas, such as daily sanitation, feeding and nutrition, and physical examination to maintain captive PRCTs in good health condition. To date, the PRCT captive breeding program is the longest and successful captive breeding program on amphibians running for over 32 years without interruptions (D. Barber, AZA, 2013, pers. comm.; NotiCel 2013).

The AZA Species Survival Plan (SSP) coordinator maintains an international studbook for the crested toad population and all animals are individually identified by an International Species Information System (ISIS) and studbook number (USFWS 2013b). Moreover, all PRCTs in the captive population are marked with passive integrate transponder (PIT) tags and individual institutional records are kept, as well as health records. Routine health checks are performed on captive toads throughout the year (i.e., fecal, blood, chytrid, ranavirus, etc.), or as needed at individual institutions and adult toads are screened prior to breeding events for release of offspring to the wild. This information is used to determine cause of death, nutrition needs (e.g., Vitamin A deficiency) and overall health status of the captive population. It is also to help on the establishment of normal baseline values of a healthy population, and help direct treatment regimens, husbandry, and nutritional parameters.

The number of individuals in captivity may fluctuate through time due to reduction by natural mortality and expansion due to new recruitments. Recent information on the genetic study reveals that northern and southern populations have no significant genetic differences as previously thought (Beauclerc et al. 2009); therefore, both demes have been crossed since 2009 (Barber 2009). In 2009, the number of toads per population was as follow: Northern: 68 males, 9 females, and 79 juveniles for a total of 156 toads in 7 institutions; Southern: 135 males, 156 females, and 246 juveniles for a total of 576 toads in 21 institutions; and Northern x Southern = 39 juveniles at Fort Worth Zoo (Barber 2009). In 2013, about 602 toads (203 males, 155 females, and 244 juveniles) were in captivity in 31 Zoo institutions (Barber 2013). Presently, the PRCT population in captivity consists of about 900 toads, including reproductive toads (females and males) from southern population and progeny from the crossing of northern and southern population. Unfortunately, the number of female PRCTs from the northern population has declined at the point that this deme no longer will have pure lineage in the captive population (D. Barber, AZA, 2014, pers. comm.).

The facilities currently housing crested toads include: Buffalo Zoo, Central Park Zoo, Cleveland Metro-parks Zoo, Dallas Zoo, Detroit Zoo, Fort Worth Zoo, Granby Zoo, Louisville Zoo, Lowry Park Zoo, Marwell Zoo, Miami Metro Zoo, North Carolina Zoo, Oklahoma city Zoo, Potter Park Zoo, Pueblo Zoo, Sedgwick County Zoo, Saint Louis Zoo, El Paso Zoo, Santa Barbara Zoo, Nashville Zoo, Omaha Zoo, Tampa Zoo, San Antonio Zoo, Toledo Zoo, Toronto Zoo, and Granby Zoo.

From 1992 to present, approximately 312,044 tadpoles and 1,546 toadlets have been produced in captivity and released into the wild at seven locations in Puerto Rico (Table 2). In 2013, over 71,079 captive breed tadpoles and 520 toadlets were produced by AZA to be released in five sites in Puerto Rico (Barber 2013). By 2014, over 252,703 tadpoles from the southern population have been released at three sites in southern Puerto Rico; and 59,341 tadpoles from the northern population and crossing of northern x southern population, have been released at three sites in the northern karst region of Puerto Rico. In addition, 1,546 toadlets produced in captivity were released at Manglillo Grande and El Tallonal. The first re-introduction effort of PRCT was conducted in 1982 in the municipality of Quebradillas, releasing 75 toadlets.

# 2. Five Factor Analysis -

# (a) Present or threatened destruction, modification or curtailment of its habitat or range:

When the PRCT was listed in 1987, the USFWS identified Factor A (modification and destruction of habitat) as one of the most important threats to the species (52 FR 28829; USFWS 1992). The recovery plan states that the species' habitat in Puerto Rico has been largely modified or destroyed through deforestation, agricultural practices and urban development, thus eliminating the species throughout most of its former range (USFWS 1992). Currently, some agricultural practices and development of residential, commercial, and touristic projects remain as threats to the species, and we believe some of these sources have been responsible for elimination of some individuals and breeding habitat.

The amount of protected habitat for the PRCT in the northern karst of Puerto Rico has increased since 1992, but the availability and suitability of that habitat in non-protected areas is decreasing (A. Vale, CDK, 2008, unpubl. data). The suitable habitat for the species in the northern karst is fragmented by urban development and agricultural practices. An analysis of land-use in the municipality of Quebradillas by Roseanne Medina (2004, unpubl. report *in* Ross 2007) showed a decrease of 29% in grass areas and agricultural land from 1963 to 2001, whereas urban development increased by 21% (Figure 8). Fewer corridors to the breeding ponds are available as urban development increase, eventually cutting off access to breeding sites (Ross 2007). The historical breeding sites have been filled or drained for construction, cultivation, and mosquito control. In 2009, the González pond, a manmade pond were PRCTs were last observed in 1992, was destroyed for mosquito control (Estremera, Liga Ecological de Quebradilla, 2010, pers. comm.).

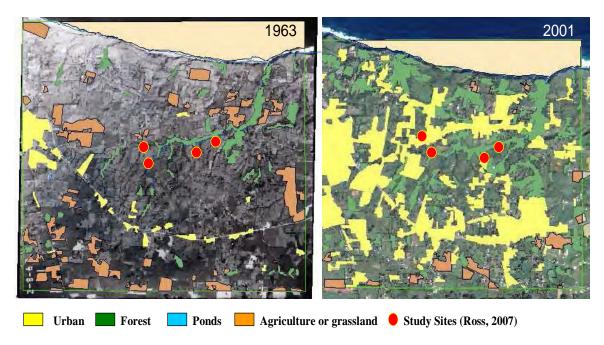


Figure 8. Comparison of land use changes in the municipality of Quebradillas, Puerto Rico, from 1963-2001 as viewed through satellite images and analyzed in ArcView 3.2 by Roseanne Medina (2004, unpubl. report *in* Ross 2007).

At the GCF, the PRCT natural breeding ponds and the introduction site in Manglillo Grande are located adjacent to at least one of the following manmade features: recreational areas, roads, and trails. The primary PRCT natural breeding ponds (i.e., Atolladora, Aroma, and Tamarindo) are located adjacent (less than 90 feet (30m)) to road PR 333, a public paved road that provides access to Atolladora and Tamarindo beaches (Figure 4). Both the Atolladora and Tamarindo breeding ponds are located adjacent (less than 164 feet (50 m)) to these beach areas, which are visited year round by tourists and local people for recreation (Pacheco, USFWS, pers. obs). The Tamarindo pond is the most significant PRCT breeding pond in the GCF, and is located on a salt flat that during dry season, about 50 percent of the salt flat area is used as parking by visitors. During heavy rain events, when the parking lot gets flooded, an intermittent shallow fresh water lagoon is developed and is frequently used by PRCTs for reproduction. Furthermore, the Mesetas trail runs over the Tamarindo breeding pond, and the Cuevas trail runs nearby (less than 164 feet (50 m)) to the western edge of the pond. These trails provide free access to the public to natural and scenic areas in the GCF. The Manglillo Grande release pond also is located close (less than 75 feet (25 m)) to an area frequently used by local people for camping and recreation.

Presently, the effects on the survival of the PRCT from the existing road, parking lot, touristic areas, and trails around the breeding ponds in the GCF are not well understood. However, we believe that any improvement to the parking facility in Tamarindo beach, widening or increase in traffic on road PR 333, and any increase in recreational use in Manglillo Grande, may adversely affect the suitability of these breeding ponds and its surrounded habitat. As well, intensification in visitation to the Mesetas and Cuevas trails, couple with habitat fragmentation (e.g., opening of new trails, changes in vegetation structure by human

trampling), and high vehicle use of the roads, may negatively impact PRCT toadlets and adults during breeding events (e.g., migration) and its habitat through loss of connectivity, direct mortality, edge effects and change in hydrology.

At Gabia Farm, the PRDNER is managing 155.38 acres (62.88 ha) for conservation, 77.69 acres (31.44 ha) of this land have been enhanced for the PRCT by reforestation and wetland creation (J. Casanova, PRDNER, 2010, pers. comm.). However, suitable habitat for the species surrounding Gabia is subjected to agricultural practices and urban development (Figure 5). Based on our experience, the land to the north of Highway PR 52 is currently under urban development pressure. According to the Puerto Rica Planning Board, 11 development projects have been proposed around the Gabia Farm, which have the potential to affect around 1,714.86 acres (693.98 ha) (PRPB, 2010, www.jp.gobierno.pr). Urban development adjacent to the Gabia Farm will fragment the habitat, limiting the PRCT population expansion in the area.

In Punta Ventana, the PRCT population occurs within an area currently proposed for the construction of a wind mill project known as San Francisco Wind Farm (SFWF). The wind mill project area consists of 79 ha (195.2 acres) of dry forest habitat where a total of 5.1 ha (12.6 acres) will be removed temporarily and eventually re-vegetated, and 0.7 ha (1.7 acres) will result in permanent habitat loss (Powell and Guarnaccia 2013; USFWS 2013a). Since the discovery of the species in Punta Ventana, the landowner of this property has expressed his interest in protecting this population (Powell and Guarnaccia 2013; USFWS 2013a). Thus, USFWS has been providing technical assistance to the landowner and conservation measures have been developed to avoid or minimize possible adverse effects on the species (USFWS 2013a). In 2013, the SFWF amended an existing Habitat Conservation Plan to include the PRCT and included conservation measures for the species. Then, the USFWS issued an Incidental Take Permit (TE104073-2) to SFWF for incidental take of the PRCT (USFWS 2013a). Presently, the proposed project will only impact PRCT upland habitat and will not impact the breeding pond. This pond is located within the proposed conservation area.

Since 1985, the Ciénaga wetland has been recognized by the PRDNER and the USFWS as a natural PRCT breeding site in the southern karst of Puerto Rico (Moreno and Canals 1985; USFWS 1992). This wetland is adjacent (less than 100 meters (358 ft)) to the Ciénaga Community in Barinas Ward, Yauco. In 2010, Service biologists documented several individuals and egg masses of this species in the wetland area after a heavy rain event (Pacheco and Zegarra, USFWS, 2010, unpubl. data). Currently the PRCT in Ciénaga wetland requires special management consideration to protect the species and its habitat from threats posed by human activities (e.g., channelization and earth movement, development, pollution). These threats may result in changes in the habitat, abundance of predators and competitors in and around the PRCT habitat, and degradation of water quality from illegal garbage dumping, household practices (e.g., car washing), disposal of untreated sewage, and agricultural practices (e.g., use of herbicides, fertilizers, or insecticides). Presence of garbage generated by people has been documented within drainages and the wetland (C. Pacheco, USFWS, 2009, pers. obs.), attracting potential predators and diseases for the PRCT.

The Ciénaga breeding pond is situated in the floodplain of an intermittent stream that drains a large area of the GCF karst hills. The soil at the floodplain is composed of Vayas silty clay (VaA), which is listed by the Natural Resources Conservation Service as hydric due to ponding (USDA 2014). This soil is poorly drained and has slow permeability, resulting in longer flooding periods (USDA 2014). The site may also be spring fed since some water is seen flowing out of the road bank on the northern side. During heavy rain events, the volume of water spreads out over the wetland area, often affecting the Cienéga residents, and temporarily interrupting the access to the community. In an effort to allow for faster drainage of flood waters, PRDNER widened and deepened the natural stream channel in the south and constructed another shallow channel on the north side of the wetland. Sediments excavated from the channels were deposited into the wetland area at various points.

The USFWS recognizes that flooding may represent a security issue for the community and does not object that maintenance activities be performed to alleviate the flooding issue. However, periodical removal of riparian vegetation and dredging activities may facilitate drainage of the wetland and accelerate colonization of invasive herbaceous vegetation (e.g., *Typha domingensis*), affecting the suitability of the pond. Additionally, deposit of sediment excavated may reduce the capacity of the wetland to hold water of the floodplain and may increase the water overflow velocity, resulting in washout of PRCT eggs and tadpoles.

Presently, vegetation on the area is mainly represented by southern cattails (*Typha domingensis*) and leather fern (*Achrostichum spp.*). Invasive native wetland plants such as Southern cattail may occupy and alter diverse native wetland communities, often resulting in plant monocultures that support fewer wildlife species (Houlahan and Findlay 2004). Southern cattail may alter the wetland attributes, including geomorphology, fire regime, hydrology, microclimate, nutrient cycling, and productivity (Woo and Zedler 2002). Based on our previous experience in the Laguna Cartagena National Wildlife Refuge, the southern cattail colonized disturbed areas faster than other native wetland plants, thereby excluding the native plants. Southern cattail is currently found growing densely in shallow areas of the Ciénaga wetland, creating a thick wall that may obstruct the access to PRCTs for breeding (C. Pacheco, USFWS, 2014, pers. obs.). If the cattail continues spreading and colonizing, it will affect the suitability of this breeding habitat.

The species' rarity and restricted distribution make it vulnerable to habitat destruction and modification. Sources of habitat destruction and modification include some agricultural practices, residential, industrial and commercial development, and elimination of natural ponds due to flood control projects and mosquito control. Therefore, we believe that Factor A continues to be a threat to the species. However, we consider the severity of this threat as moderate and low because most of the known populations occur on protected lands managed for conservation.

# (b) Over-utilization for commercial, recreational, scientific, or educational purposes:

When the recovery plan for the PRCT was approved, this factor was identified as a threat. We have found no information regarding the illegal hunting or collection of the PRCT for

commercial, recreational, scientific or educational purposes. Therefore, we do not believe this factor is a threat to the PRCT at this time.

# (c) Disease or predation:

At the time of listing, predation and disease had not been documented as factors for the decline of the PRCT (USFWS 1992). However, the recovery plan states that predation by the common anole (*Anolis cristatellus*), Puerto Rican ground lizard (*Ameiva exsul*), and birds could become a significant factor if PRCT populations are greatly reduced by other factors. In addition, the plan suggests feral dogs (*Canis lupus familiaris*) and cats (*Felis catus*), mongoose (*Herpestes auropunctatus*) and the giant marine toad (*Rhinella marinus*) as potential predator for the PRCT (USFWS 1992).

The introduced giant marine toad had been identified as possible predator for the PRCT, affecting its distribution and abundance. However, authors such as Rivero (1980) and Beauclerc (2009), suggested that the PRCT was rare prior to the introduction of the marine toad and that it cannot be solely blamed for the toad's current scarcity. Rivero (1980) also stated that the PRCT and the marine toad can coexist at the same place. However, marine toads have been observed competing with the PRCT for shelter around the breeding pond (M. Canals, former GCF Manager, PRDNER, 2009, pers. comm.). In fact, Flores-Vallejo (former graduate student from the Metropolitan University, 2011, unpubl. data) documented a marine toad eating a PRTC toadlet at El Tallonal. This information suggests that marine toads compete for food, space and can depredate on the PRCT.

Johnson (1994) reported that mongoose predation was a significant factor on the survival of captive raised toads released at the GCF. M. Canals (former CGF Manager, PRDNER) further stated that predation on dispersing toads may be heavy, particularly from wading birds (USFWS 1992). He observed seven PRCT females killed by a Blue heron (*Ardea herodias*) during a single breeding event at Tamarindo (M. Canals, former CGF Manager, PRDNER, 2008, unpubl. data). In addition, Sondra Vega (IH, 2009, pers. comm.) observed a bull frog (*Rana catesbeana*) predating on PRCT tadpoles in the artificial ponds at El Tallonal.

In 2008, several AZA institutions reported Vitamin A deficiency problems with their toads, most of which were offspring from a single clutch (D. Barber, AZA, 2008, unpubl. data). Initial symptoms included weight loss and failed ability to capture prey items. The inside of the mouth in several specimens contained brown, crusty material on the roof of the mouth and tongue. Several toads died and histopathology confirmed *Squamous metaplasia* on the tongue and a diagnosis of Hypovitaminosis A was confirmed at four institutions. Once the problem was identified, most toads were successfully treated with oral and topical doses of Retinol A (D. Barber, AZA, 2008, unpubl. data). Treatment regimens and powdered vitamin supplements are currently being evaluated to reduce the risk of deficiencies in the future.

Recently, the pathological effect of chytrid fungus (*Batrachochytrium dendrobatidis*) has been identified as a possible threat to the PRCT (Crawshaw 2007). Chytrid fungus has been attributed to the possible extinction of at least three coqui frog species (*Eleutherodactylus spp*) in Puerto Rico (Longo and Burrowes 2010; Barber 2011). Fieldwork involving amphibian

monitoring and chytrid fungus detection at various sites on the island, suggest that chytrid is abundant through the highland forests of Puerto Rico at elevation of about 1,940 feet (600 m) (Joglar et al. 2007). This range is outside of the known PRCT locations. According to Burrowes et al. (2008) the absence of chytrid fungus at low elevations in Puerto Rico might be explained by the high diurnal temperatures which are often above the thermal tolerance reported for this fungus. However, Barber (2011) detected the presence of chytrid fungus in two sites below 656 feet (200 m) in elevation in Puerto Rico. From 2006 to 2011, Barber collected a total of 157 samples from marine toads, white-lip frog (Leptodactylus albilabris), some coqui species, and the PRCTs at seven locations within the historical and current distribution of the PRCT (i.e., Quebradillas, Arecibo, Río Encantado, Gabia, Manglillo Grande, Punta Ventana, and Tamarindo) (Barber 2011). All but two samples were negative for chytrid fungus. These two samples rated as "strongly positive" (Barber 2011). Chytrid fungus was confirmed in an Eleutherodactylus antillensis in 2010 from Río Encantado, at elevation of 630 feet (192 m) from sea level. The second chytrid-positive sample was collected from an *Eleutherodactylus coqui* at El Tallonal in Arecibo at elevation of 269 feet (82 m) from sea level.

Preliminary studies of skin peptides in a small sample of captive PRCT suggest that they do not possess peptides with antimicrobial activity (Barber 2011). Peptides are short chain of amino acid monomers that allow the creation of antibodies in animals. Although these peptides often are present in species of amphibians that exhibit a natural immunity to chytrid fungus infection, not all species secrete them (Rollins-Smith and Colón 2005). In 2012, Dr. Ryan DeVoe, Veterinarian from North Carolina Zoological Park, conducted preliminary study on the effect of chytrid fungus on PRCTs, discovering that the species is resistant to the pathogen while they are kept at around 20-26°C (68-70°F) (R. DeVoe, 2012, unpubl. data). Although PRCT individuals continue to be monitored for the chytrid fungus pathogen, no other disease factors are currently known to be affecting the PRCT.

Presently, known PRCT predators are marine toads, bull frogs, mongoose, and blue herons, among others. As well, some diseases may affect PRCT. However, the overall effect of disease and predation on the species is speculative as no information is available to relate a population decline to these factors. Nevertheless, disease and predation could contribute to an additive mortality should PRCT populations become greatly reduced by other factors. We believe that Factor C is low in severity and non-imminent to the species because only anecdotal evidence has been found suggesting that PRCT is threatened by disease or predation.

#### (d) Inadequacy of existing regulatory mechanisms:

When the PRCT was listed, the inadequacy of existing regulatory mechanisms to protect the species was not identified as a threat. Following listing, the PRCT acquired protection under the Endangered Species Act of 1973, as amended. Currently, PRCT is protected by the Puerto Rico Commonwealth Law No. 241, known as the New Wildlife Law of Puerto Rico (*Nueva Ley de Vida Silvestre de Puerto Rico*) approved in 1999. The purpose of this law is to protect, conserve and enhance both native and migratory wildlife species; declare property of Puerto Rico all wildlife species within its jurisdiction, regulate permits, hunting activities, and exotic

species among other activities. In 2004, the PRDNER approved Regulation 6766 to regulate the management of threatened and endangered species in Puerto Rico (*Reglamento para Regir el Manejo de las Especies Vulnerables y en Peligro de Extinción en el Estado Libre Asociado de Puerto Rico*). The PRCT was included in the list of protected species of this Regulation and designated as critically endangered. Article 2.06 of Regulation 6766 prohibits collecting, harassing, hunting, removing, among other activities, of listed animals within the jurisdiction of Puerto Rico. Under this article, the habitat deemed as essential to the survival of the species is also protected.

The PRCT is also protected by the Lacey Act (P.L. 97-79, as amended; 16 U.S.C. 3371 et seq.), which makes it unlawful to import, export, transport, sell, receive, acquire, or purchase any wild animal (alive or dead including parts, products, eggs, or offspring).

Despite the protection of the PRCT by existing laws, the enforcement of such laws and regulations, particularly on private lands, continues to be a challenge due to the lack of knowledge on the species by regulatory agencies, landowners, and some law enforcement officers. While the above mentioned laws and regulations are in place, elimination and modification of PRCT breeding habitat has occurred in areas where the species may have naturally occurred. Moreover, permits to implement agricultural practices (e.g., deforestation for grassland and use of pesticides for pest control), and urban development within and near PRCT natural populations are prevalent.

Under Factor A and E, we discussed in more detail certain cases of lack of enforcement that threatened the species and its habitat. For these reasons, we conclude that the inadequacy of existing regulatory mechanisms is a threat to protect the PRCT and its habitat.

#### (e) Other natural or manmade factors affecting its continued existence:

At the time the PRCT was listed, this factor was identified as a threat to the species. The PRCT was listed primarily due to its highly limited geographical distribution and its vulnerability to demographic and environmental catastrophes. Presently, the species continues to be threatened by this factor and it is of conservation concern due to its limited breeding sites, within its small geographic range.

The PRCT is vulnerable to extinction due to its low reproductive population number (one natural population) and its limited distribution, coupled with habitat loss or alteration. Presently, the primary natural breeding ponds are located adjacent to at least one of the following manmade features: agricultural lands, recreational areas, roads, trails, homes, or other manmade structures.

The proximity of a parking area, trails and an access road to the most significant breeding ponds for the PRCT augments the risk of road fatalities and increases encounters with humans during reproductive events. Vehicle traffic on roads within the essential habitat of amphibian species can be a direct source of mortality and, in some instance, can be catastrophic and should not be underestimated (Glista et al 2007). Increase of vehicle traffic and human trampling through or close by the breeding pond areas may affect the survival of tadpoles,

toadlets and sometimes adult toads during its migration to and from the pond during breeding events. Although the PRDNER limits the vehicle access to the Tamarindo beach when the area is flooded, and during PRCT breeding events, the public still have access on foot, on bicycles, and sometimes on vehicles through the breeding pond to the beach area and trails. As well, the public is allowed to park their vehicles about 100 feet (30.5 m) from the edge of the pond and along the road PR 333. Road kills and squashing by human of newly metamorphosed toads and toadlets are much more likely during daylight when they are more active, and the number of visitors at this site also increases. Road kills of adult and sub-adults are more likely at night when they are more active and difficult to see on the road. Therefore, we believe that any increase in vehicle traffic and visitation to the Tamarindo area and through the access road would result in loss of significant portion of toads during breeding events, and may adversely affect the suitability of this breeding habitat.

Vehicular traffic is associated with a wide variety of contaminants including Polycyclic Aromatic Hydrocarbons (PAHs) from incomplete combustion, exhaust, oil leaks, tire abrasion, asphalt, and other lubricant (cita). PAHs have been linked with many undesirable health consequences in human and animals, and exposure to amphibians may cause broad effects as increased mortality, genotoxicity, larval deformities, histological changes to the integument, slowed development, and larval hyperactivity (ENSR 2004; Gjeltema et al. 2012). A recent study by Gjeltema et al. (2012) revealed that diverse population of PAH analyses were found in high concentration within the Tamarindo parking lot, which as expected, is associated with higher levels of vehicular traffic. However, as adverse effects of PAHs on PRCTs have not been documented, we consider this threat as speculative at this point.

Use of pesticides, herbicides and chemical for mosquito control may adversely affect the suitability of breeding ponds located adjacent to urban developments and agricultural lands (USFWS 1992). Ponds historically used by the PRCT for reproduction in Quebradillas were drained for mosquito control.. Nearby neighbors also chlorinated these ponds to limit the production of mosquitoes (Ross 2007). Rain and runoff from grasslands commonly sprayed with herbicides, chemical fertilizers and pesticides would also add to possible contamination of the breeding ponds. Many studies have documented negative impacts of agrochemicals on amphibians; impacts include deformities, abnormal immune system function, diseases, injury and death (Reeder et al. 1998; Davidson et al. 2001; Hayes et al 2002). Therefore, we believe that the PRCT could be affected by agrochemical practices and mosquito pest control. However, this threat should be considered low in its magnitude and imminence because the primary breeding ponds known at this time are located in lands managed for conservation.

The species' breeding requirements and limited distribution may exacerbate its vulnerability to natural events (i.e., long periods of drought) and anthropogenic events (i.e., induced wild fires, pest control) compromising the continued existence of the PRCT.

Changes in climate can have a number of direct and indirect impacts on species, and can exacerbate the effects of other threats. Rather than assessing climate change as a single threat in and of itself, we examined the potential consequences to species and their habitats that arise from changes in environmental conditions associated with various aspects of climate change. Vulnerability to climate change impacts is a function of sensitivity to those changes, exposure

to those changes, and adaptive capacity (IPCC 2007; Glick et al. 2011). An expected effect of the climate change is the increase of sea level and on the intensity of hurricanes and tropical storms, followed by extended drought periods (IPPCC 2012).

The recovery plan did not reference sea level rise as potential threat to the PRCT. The natural breeding populations located at Tamarindo and Punta Ventana are extremely vulnerable to storm surges cause by hurricanes and to sea level rise due to its proximity to the sea. Increase in sea level may affect the species and the habitat on which the species depend for reproduction at these two sites. The two breeding ponds are located on coastal areas with a maximum elevation of less than 3 feet (1 m), and are less than 100 meters (328.08 ft) from the sea. Recent studies revealed that over the past 100 years, the globally-averaged sea level has risen approximately 10 to 25 centimeters (Rahmstorf et al. 2007), a rate that is an order of magnitude greater than that observed in the past several thousand years (Douglas 2001 in Hopkinson et al. 2008). The IPCC (2007) suggests that by 2080, sea level rise could convert as much as 33 percent of the world's coastal wetlands into open water. Although rapid changes in sea level are predicted, estimated time frames and resulting water levels vary due to the uncertainty on global temperature projections and the rate of ice sheets melting and slipping into the ocean (IPCC 2007, CCSP 2008). Thus, because the change in sea level is a long term process and may occur a long period of time, the scope of this threat should be considered as moderate.

Hurricanes, storms and heavy tidal waves should be considered threats to the PRCT. Breeding ponds, hence populations, could easily be lost if the narrow sandy beach barrier is destroyed storm surge and pools are flooded with sea water during severe hurricanes and tropical storms, which also usually set the conditions that triggers breeding events (Lentini 2003; M. Canals, former CGF Manager, PRDNER, 2008, unpubl. data). In fact, M. Canals witnessed PRCT breeding pairs being washed out to sea when a pond in Atolladora was inundated during a hurricane in 1985 (Johnson 1990). In August 2007, during Hurricane Dean, seawater entered into the Tamarindo breeding pond and PRDNER had to add 30,000 gallons of freshwater to decrease salinity levels to less than 9 ppt in order to save developing tadpoles (Barber 2007).

Intense heavy rains followed by prolonged drought period also may affect PRCT populations, especially since reproduction in this species appears to rely on climatic events. PRCT reproduction events are triggered by intense heavy rains. But the survival rate of the new metamorphosed toads and toadlets during prolonged drought period after a breeding event is unknown, even when adaptation to dry environment is expected. New metamorphosed toads and toadlets are probably the most vulnerable stage of the PRCT development as they are developing terrestrial skin gland, thus are more susceptible to desiccation, as their lungs, heart and aerobic capacity are still not fully developed (McDiarmid and Altig 1999).

Hurricanes followed by extended periods of drought may result in changes in soil and microclimate conditions, and may allow other species (native or non-native) adapted to drier conditions to become established (Lugo 2000). Invasive plant species (e.g. *Megathyrsus maximus*) may spread and colonized PRCT habitat, altering the habitat and nutrient cycling, and promoting fires that would affect this species (Ammondt and Litton, 2012).

The known PRCT populations at the GCF, Punta Ventana, and Gabia are located within the driest zone of Puerto Rico, which is susceptible to human-induced adverse impacts such as fires. The rapid growth of grasses increase becomes a fuel for fires. J. Casanova (PRDNER, 2013, pers. comm.) reported at least one human induced fire per year affecting not less than 5 acres (2.02 ha) in the privately owned lands around Gabia Farm. At the GCF, M. Canals (PRDNER, 2013, unpubl. data.) reported several (1 to 4) human induced fires per year, affecting not less than 1 acre (0.40 ha.) along road PR 333, and the Tamarindo beach area. Even when PRDNER implements a fire-prevention and management program during dry season, we believe this factor should still be considered as a threat because of the distribution of the PRCT in dry areas and the susceptibility of some occupied areas to human activities and wildfires.

Overall, we consider the effects of hurricanes, prolonged drought periods, wild fires, use of agrochemicals, human trampling, and visitation as current threats to the PRCT. The population dynamic of the species is poorly known (e.g., survival, competitive ability during early life stages), there are only few known natural populations, and there is a lack of information to determine what constitutes a viable population. We consider the severity of above mentioned threats as moderate because the effect of each threat is not expected to affect all populations at once. As well, we considered them to be low imminent because threats like climate change are not likely to occur immediately.

# **Synthesis**

The PRCT was listed as a threatened species in 1987, due to its restricted and fragmented distribution, increase of exotic predators (i.e., mongoose, cats and amphibians such as cane toads), and habitat disturbance. Subsequently, the Recovery Plan for the PRCT was approved in 1992. At that time, the species was considered to have a high degree of threat, but a high recovery potential. In 1992, around 2,050 PRCT individuals were reported in two populations in Puerto Rico: GCF (2,000 toads) and Quebradillas (25 to 50 toads).

Since the recovery plan was approved, extensive searches have been conducted throughout the historical range of this species. Presently, the PRCT is exclusively known from Puerto Rico, where it is known from nine localities. Three are natural populations: GCF, Punta Ventana in Guayanilla, and Ciénaga in Yauco. The other six populations are on reintroduced sites: Manglillo Grande, El Tallonal, Río Encantado, La Esperanza, Gabia Farm, and Cueva El Convento. The GCF, Manglillo Grande and Gabia Farm sites are managed by PRDNER. Whereas, El Tallonal, Río Encantado, and La Esperanza are private lands managed for conservation by PLN. Punta Ventana is a private land subjected to the construction of a wind mill project. Nevertheless, the landowner has expressed interest in protecting the species and developed a Habitat Conservation Plan with conservation measures for this and other listed species. The Virgin Gorda population in BVI is considered extirpated.

The conservation of the wild PRCT requires a detailed understanding of its populations (i.e., size, spatial distribution, and demographic trends). Presently, no standardized quantitative population estimates are available for the PRCT. Only toad counts during reproductive

events, and number of tadpoles released during reintroduction efforts are available. Presently, about 312,044 tadpoles and 1,566 toadlets have been released at the six re-introduction sites.

The PRCT population at the GCF has been considered stable during the last decade, recording up to 2,224 toads in a single breeding event. A new natural PRCT breeding pond was discovered in Punta Ventana, Guayanilla. In addition, the species was rediscovered in Ciénaga wetland, Yauco. The PRCT status at Manglillo Grande, Punta Ventana, El Tallonal, Gabia, Río Encantado and La Esperanza is improving since toads are frequently sighted at these sites. Based on the new information, we believe the overall population status of PRCT is increasing.

Since 1982, AZA has maintained healthy PRCT individuals in captivity. At the beginning the northern and southern PRCT populations were managed separately in captivity because preliminary genetic data suggested the two populations had significant differences that reflected their geographical separation. In 2010, a study on the genetic variation among the PRCT populations found that the northern population is moderately divergent from the GCF population to the point that does not warrant classification as separate subspecies. As well, the study revealed that the southern population, both in captivity and in the wild, has not suffered reduced genetic diversity. But, the northern population may not be sustained much longer because of the low number of individuals in captivity descending from four inbred siblings. Based on the new genetic information, species experts recommend that a third breeding colony be established in which northern and southern individuals be combined to ensure the persistence of some northern traits in the event that the pure northern linage become extinct due to demographic stochasticity or inbreeding depression.

Presently, the PRCT population in captivity consists of about 900 toads including reproductive toads from southern population and progeny from the crossing of northern and southern population. Unfortunately, the PRCTs from the northern population have declined to the point that this deme is no longer a pure linage in the captive population.

Currently, the PRCT is found in subtropical dry forest and subtropical moist forest. These two forest types are found widely distributed in Puerto Rico and USVI. The new information suggests that habitat availability is not a constraint to the species. Therefore, we believe that additional toads may be in other karst areas in Puerto Rico and USVI.

The PRCT recovery plan contains criteria for delisting: the maintenance of a stable or growing population of PRCT at six locations during a 10 year period; and maintain five captive populations. Based on the information gathered and analyzed during this review, these criteria have been partially met. The PRCT populations located at the GCF, and Punta Ventana have been considered sustainable because reproduction and migration of toadlets have been documented. Furthermore, the PRCT was successfully established in Manglillo Grande where breeding events have been documented. At El Tallonal, Río Encantado, La Esperanza, Cueva El Convento, and Gabia Farm, PRCTs are frequently sighted, but no successful breeding events have been documented.

According to the five factor analysis conducted for this review, the PRCT is threatened by Factor A (present or threatened destruction, modification, or curtailment of it habitat and range), Factor C (Disease or predation), Factor D (Inadequacy of existing regulatory mechanisms) and Factor E (Other natural or manmade factor affecting its continued existence). Natural and human factors such as urban and tourist development, non-native predators (i.e., mongoose, cane toad, blue heron, and bull frog), habitat modification by catastrophic events (i.e., hurricanes, storms, climate change, and sea level rise), and lack of enforcement of existing laws and regulations pose a threat to the PRCT and its habitat.

Each of the threats mentioned above represent a variety of direct and indirect impacts on the species, and can exacerbated the effects of the others threats. Hence, we evaluated the potential consequences on the species and its habitat rather than assessing each one as a single threat. Overall, we consider the severity of these threats as moderate because they are expected to affect only some individuals or some populations instead of the entire populations or range. As well, we considered these threats as low in immediacy because most of the threats are not likely to occur in near future.

The ESA defines a threatened species as any species which is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range. Therefore, based on the information gathered during this review, we believe that the PRCT continue to meet the definition of threatened.

#### III. RESULTS

Α.	<b>Recommended Classification:</b>
	Downlist to Threatened
	Uplist to Endangered
	Delist
2	X No change is needed

# B. New Recovery Priority Number \_8c\_

Based on the information gathered during this review, we recommend the new recovery priority number of 8c, which indicates the species faces a moderate degree of threat and a high recovery potential. Additionally, the recovery of the species was considered to have, or may have conflicts with construction or other development project or other form of economic activity, therefore the letter "c" is added.

### IV. RECOMMENDATIONS FOR FUTURE ACTION-

Based on the best available information, we recommend the following actions:

- Revise the recovery plan to include new information on the species and develop new and up-to-date measurable criteria for delisting the species.
- Conduct quantitative efforts to estimate relative abundance of the species at all known populations.
- Initiate the mark and recapture study to establish confident population estimates.
- Conduct additional surveys in traditional and nontraditional areas with suitable habitat for the species in PR, USVI and BVI, including St. John and Vieques Island, to determine the range of the species.
- Refine habitat description and suitability based on GAP analysis and other geographical related mechanisms.
- Continue to support predator eradication (mongoose, cane toad, and bull frog) on breeding areas and other PRCT habitat.
- Develop public education and outreach programs for the PRCT at the entire northern karst and southern karst in PR, and at Virgin Gorda, BVI.
- Develop cooperative agreements with local government and private landholders for the conservation and protection of suitable habitat for the PRCT in PR and BVI.
- Tamarindo and Punta Ventana ponds continue to be the most important breeding sites for the species. Efforts must be taken to continue protecting these ponds from salt water intrusion. Also, develop alternatives to assist the migration of the species to higher areas not subject to salt intrusion.
- Develop and implement strategies to protect and conserve the PRCT natural population at Ciénaga in Yauco.
- Continuing with re-introduction efforts.
- Reestablish the PRCT captive breeding program in PR.

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# U.S. FISH AND WILDLIFE SERVICE 5-YEAR REVIEW of Puerto Rican crested toad / Peltophryne lemur

Threatened
Recommendation resulting from the 5-Year Review
X No change is needed
Review Conducted By Carlos Pacheco, Caribbean Ecological Service Field Office, Boquerón, Puerto Rico.
FIELD OFFICE APPROVAL:
Edwin E. Muñiz, Lead Field Supervisor, U.S. Fish and Wildlife Service  Approve  Approve  Date  9/n/2015
REGIONAL OFFICE APPROVAL:
Lead Regional Director, U.S. Fish and Wildlife Service  Approve La Ellis Date 6/21/2016
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#### APPENDIX A

# Summary of Peer Review for the 5-Year Review of the Puerto Rican crested toad (*Peltophryne lemur*)

- **A. Peer Review Method:** A draft 5-year review was sent to each of the following experts, as an attachment to an email, requesting their review and any other changes or additions that should be included in the document. All reviewers have extensive knowledge of this and similar species.
  - 1. Dr. Miguel A. Garcia, Director, Bureau of Fisheries and Wildlife, Puerto Rico Department of Natural and Environmental Resources, San Juan, Puerto Rico. E-mail: mgarcia@drna.gobierno.pr
  - 2. Dr. Alberto Puente-Rolón. Herpetologist, Iniciativa Herpetológica, Arecibo, Puerto Rico. E-mail: albertonski@hotmail.com
  - 3. Ms. Diane Barber, Coordinator Puerto Rican crested toad Species Survival Plan, AZA, Fort Worth Zoo, Fort Worth, Texas. E-mail: dbarber@fortworthzoo.org
  - 4. Mr. Miguel Canals, Former Manager for the Guánica Commonwealth Forest, PRDNER, Guánica, Puerto Rico. E-mail: menqui@hotmail.com
- **B.** Peer Review Charge: Reviewers were charged with providing a review of the document, including any other appropriate comments and/or additions. Reviewers were not asked to comment on the legal status of the species.
- **C. Summary of Peer Review Comments/Report:** Reviewers responded by email. All reviewers thought the information in the document provided to them was accurate.
- **D. Response to Peer Review:** Recommendations from the reviewers were incorporated into the document as appropriate. These consisted primarily of additional information concerning the status of certain populations, threats to the species, and recommendations for future actions.