NWRC FINAL REPORT

July 30, 2017

STUDY TITLE:

Assessment of bait density, bait availability, and non-target impacts during an aerial application of rodenticide to eliminate invasive rats on Desecheo Island, Puerto Rico

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LABORATORY PROJECT ID:

QA-2588

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<u>This report should be cited as follows:</u>

Shiels, A.B., G.W. Witmer, C. Samra, R.S. Moulton, E.W. Ruell, J.R. O'Hare, J.D. Eisemann, S.F. Volker, D.A. Goldade. 2017. Assessment of bait density, bait availability, and non-target impacts during an aerial application of rodenticide to eliminate invasive rats on Desecheo Island, Puerto Rico. Final Report QA 2588. USDA, APHIS, WS, NWRC, Ft Collins, CO. 87 pp.

Summary

1. Invasive rats (*Rattus* spp.) are among the most damaging species to native biota on islands. The use of rodenticides as a method to eradicate invasive rats from islands has grown rapidly during the past two decades, and most of the rat eradications on islands worldwide have used baits containing the 2nd generation anticoagulant, brodifacoum.
2. In our study, we monitored bait application rates, bait take and availability, and non-target risk following the island-wide aerial application of Brodifacoum 25-D: Conservation to eliminate *R. rattus* on Desecheo Island, Puerto Rico, in March/April 2016. This 2016 rat eradication attempt followed a rat eradication attempt, and subsequent failure, involving the same bait presented at a lower application rate in 2012 (4 years earlier). Therefore, we assessed the non-target consequences and the fate of brodifacoum residue from both the 2012 and 2016 operations.
3. Bait was applied under an EPA-approved Supplemental Label at 30-45 kg/ha (depending upon habitat) for each of two applications (March 18 and April 9) in 2016; these applications rates were 2-3 times greater than the standard Brodifacoum 25-D: Conservation label that was used in 2012.
4. Biological sampling the week prior to the 2016 rat eradication attempt revealed that brodifacoum residues can persist in parts of the food web for up to 4 years: 2 of 23 rats (*R. rattus*; 56-58 ppb), 3 of 20 ameiva lizards (*Ameiva exsul desechensis*; 27-34 ppb), 1 of 1 pearly-eyed thrasher (*Margarops fuscatus*; 134 ppb), and 1 of 10 insect samples (a cockroach sample, Order Blattodea, of 18 individuals; 45.5 ppb) had detectable levels of brodifacoum residues. Tissue residue analyses were conducted on samples at USDA NWRC (Colorado) using liquid chromatography-tandem mass spectrometry.

5. After the 2016 application commenced, monitoring cameras revealed that rats and hermit crabs were almost exclusively the sole animals consuming the bait, and no rats were seen after 6 days following the first application. Carcass searches revealed the following dead animals post-application: 22 rats, 12 birds (10 pearly-eyed thrashers, 1 gray kingbird (*Tyrannus dominicensis*), 1 belted kingfisher (*Megaceryle alcyon*), 1 ameiva, 1 eel (unknown taxonomy), 1 hermit crab (*Coenobita clypeatus*), and 1 rhesus monkey/macaque (*Macaca mulatta*). Only a portion of the total non-target carcasses were tested for brodifacoum residues, and 22 rats, 8 birds (7 pearly-eyed thrashers, 1 kingfisher), 1 ameiva, and 1 hermit crab all tested positively for brodifacoum residues, and therefore, most likely died from primary or secondary exposure to brodifacoum.
6. Approximately 3 weeks after first application, we also live-harvested 13 ameivas, 20 geckos (*Sphaerodactylus levinsi*), and 20 anoles (*Anolis desechensis*) for brodifacoum residue testing; all animals appeared healthy, but 65-100% of these individuals contained detectable levels of brodifacoum in their livers, which ranged from 12.4 ppb to 1100 ppb; some insects and hermit crabs also had detectable levels of brodifacoum (10.3-1580 ppb).
7. Following assessments by USFWS and Island Conservation in November 2016 and April 2017, Desecheo was determined to be rat-free (announced in June 2017).
8. Although detection of residues in many animal samples was anticipated, the longevity of brodifacoum in the food web was greater than expected. Risk assessments should carefully consider entire food webs prior to operations using rodenticides. We expect that the environmental benefits (e.g., increased populations of native flora and fauna) of having Desecheo Island rat-free outweighs the risks of non-target exposure to the brodifacoum rodenticide that we have identified and quantified in our study.

Introduction

Rats (*Rattus* spp.) have been introduced to many ecosystems worldwide and are among the most widespread and problematic invasive animals affecting islands (Towns et al. 2006). Through mostly unintentional introductions by humans, these rats now occupy > 80% of the major islands worldwide (Atkinson 1985; Towns 2009). As a consequence of their omnivorous diet and large incisor teeth, introduced rats are probably the invasive animals responsible for the greatest number of plant and animal extinctions on islands (Towns et al. 2006). Of particular importance to native island biota are the threats posed by the black rat (*R. rattus*), as this rat has been ranked as the main rodent pest species globally (Capizzi et al. 2014), and particularly so for forest ecosystems on islands (Shiels et al. 2014).

Removing rats from islands has been a long-term challenge faced by land managers and conservation biologists; however, the number of islands from which rats have been removed worldwide has increased dramatically over the past several decades (Howald et al. 2007; Keitt et al. 2015), and there are now over 400 islands where rats have been eradicated (DIISE Partners 2016). Many islands are too complex (e.g., too large and/or human inhabited) for removal of all individuals of invasive rats, and therefore alternative management techniques are required to control or suppress rat populations in fragments of islands where they pose threats to natural resources, agriculture, and/or human health and safety (see recent review of various motivations, techniques, and success levels for rat control projects on islands worldwide in Duron et al. *in press*). Aerial broadcast of the 2nd generation anticoagulant brodifacoum has provided the highest success rate of eradicating rats from islands (Keitt et al. 2015), although tropical islands have had a slightly lower success rate relative to temperate islands using this same methodology (89% vs. 96.5% success, respectively; Keitt et al. 2015; Pott et al. 2015).

Dryland ecosystems are the most threatened terrestrial tropical ecosystems worldwide (Hoekstra et al. 2005; Miles et al. 2006), and they are home to many rare, threatened, or endangered species. The island of Desecheo (1.5 km^2, or 0.6 $miles^2$ total land area), off the western shore of mainland Puerto Rico in the Caribbean, is a dryland ecosystem that is composed of mostly forest. It is a U.S. National Wildlife Refuge, and it is managed by the U.S. Fish and Wildlife Service (USFWS). There are several endemic reptile species on this island, and one threatened cactus (Higo Chumbo, *Harrisia portoricensis*). Marine and terrestrial birds are relatively uncommon on Desecheo, and it is thought that the low bird numbers are due to the infestation of the black rat, *R. rattus*. Suppression of the black rat has resulted in major benefits to native and endangered species in tropical islands (e.g., Witmer et al. 2007; Pender et al. 2013). On Desecheo, *R. rattus* most-likely consumes native reptiles, birds, arthropods, and plants. The USFWS and Island Conservation (IC) had first attempted to remove all rats from Desecheo in 2012 using aerial broadcast of the rodenticide Brodifacoum-25D: Conservation (EPA Reg. No. 56228-37). The motivation for removing rats from Desecheo was to provide safe habitat for resident and non-resident birds, endemic reptiles, and native plants. Unfortunately, the rat removal operation of 2012 was unsuccessful at removing all rats, and since then, rat populations have recovered to levels equivalent to those prior to the eradication attempt. After external review of the 2012 operation (Brown and Tershy 2013), IC and USFWS approached NWRC to apply to the Environmental Protection Agency (EPA) for a supplemental label for Brodifacoum-25D: Conservation label specific to an eradication attempt on Desecheo in 2016, which would allow a higher bait application rate and a longer duration between applications compared to the main label. In November 2015, NWRC received approval from EPA for the supplemental label for use on Desecheo in 2016.

As with all projects that use toxic baits to eliminate invasive animals, we expect that there will be some negative effects to non-target organisms (e.g., see Masuda et al. 2014; Pitt et al. 2015). Justification for proceeding with such a control tool that harms some non-target species is that the longer-term effects of removing rats will provide greater benefit to the native species and habitat that goes beyond the number (and types) of non-target mortalities. Based on unpublished findings from the first Desecheo rat eradication attempt in 2012, there are no expected negative impacts to threatened or endangered species as a result of this rat eradication; there is one cactus species (*H. portoricensis*) listed as threatened under the Endangered Species Act. Expected non-target impacts may include some species being affected by eating the bait directly (primary hazard) or consuming any animal that has consumed the toxicant (secondary hazard). On Palmyra Atoll, where eradication of *R. rattus* was deemed a success by applying under a supplemental label 4-8 times the application rate brodifacoum allowed on the main label, there were 84 carcasses of fish, birds, lizards, and invertebrates that were recovered shortly after the rodenticide applications (Pitt et al. 2015). Of the subset of these animals sampled, 84% had brodifacoum residues (Pitt et al. 2015), thus demonstrating the range of non-targets and multiple levels of the food web that can be affected by island-wide rat eradications that use brodifacoum. Marine biota (e.g., fish, mussels) can also accumulate residues of brodifacoum after rat eradications, and this was recently documented on an offshore island in New Zealand that had brodifacoum bait applied to eliminate rats (Masuda et al. 2015). In a literature review of studies measuring marine biotic exposure to brodifacoum, <6% of the fish and marine invertebrates sampled had brodifacoum residues (Masuda et al. 2015). Based on unpublished findings from the first Desecheo rat eradication attempt in 2012, the terrestrial biota, particularly the lizard community, is expected to suffer from direct or indirect (via insect or rat carcass) consumption of the brodifacoum bait in the 2016 rat eradication attempt. By contrast, the marine and bird community is expected to suffer minimally from this operation given the few resident marine and terrestrial birds currently at Desecheo (K. Swinnerton, Island Conservation, personal communication) and the relatively few marine animals suffering brodifacoum exposure in other similar brodifacoum-based operations (Masuda et al. 2015).

The goal of USFWS and IC for Desecheo was to successfully remove all rats from the island in 2016. USFWS and IC conducted the operation, and therefore facilitated the application of Brodifacoum-25D: Conservation, according to the supplemental label (see below). While USFWS and IC conducted the bait application, NWRC led the monitoring efforts associated with the project. For objectivity and best practice procedures, the agency leading the operational aspects of the study should be different than those leading in the monitoring (Pitt et al. 2015). Thus, the overall objectives for this study for NWRC were to: 1) determine the distribution and density of bait from the aerial application of Brodifacoum-25D: Conservation, 2) conduct the bait take and bait availability monitoring by revisiting plots and using motion cameras to determine animals removing or consuming bait, and 3) document the non-target effects through carcass searches and analysis of brodifacoum residues throughout the consumer food web, with concentration on the lizard community.

We expected that bait would be applied according to the supplemental label and it would stay available for at least 5-7 days after application, despite the presence and abundance of non-target invertebrates such as hermit crabs that will eat a portion of the toxic bait and render it unavailable to rats (Cuthbert et al. 2012). We further anticipated that invasive rats would be removed from Desecheo using the planned application of Brodifacoum-25D: Conservation rodenticide, and that dead rats recovered after bait application would indeed contain elevated levels of brodifacoum residues. Post-application, detectable levels of brodifacoum residues were expected to be found in several parts of the non-target consumer food web, particularly within invertebrates that would be directly consuming the bait, and the lizard community that would probably gain brodifacoum exposure from consuming insects (all three lizard species measured) and dead rats (ameivas) that suffered from the poison. We expected rats, crabs, and insects to be the animals most commonly documented (using monitoring cameras) removing or consuming bait pellets, with much fewer incidences of bait consumption from lizards and birds. Based on the relatively low amounts of brodifacoum bait applied to Desecheo vs. Palmyra Atoll (e.g., Pitt et al. 2015), we expected minimal evidence of non-target mortality on Desecheo relative to Palmyra, yet higher non-target mortality in 2016 on Desecheo than found during the previous Desecheo rat eradication attempt of 2012 when 2-3 times less bait was applied (Island Conservation unpublished report). Finally, we expected there to be some legacy levels of brodifacoum residues in ameivas from the 2012 rat eradication attempt. Ameivas are predicted to be the longest lived of the three lizards included in our study, and given that residues accumulate in vertebrate tissue, we only expect some of the ameivas captured pre-application to have detectable levels of brodifacoum residues. An understanding of the effectiveness and non-target impacts of the brodifacoum baiting in the planned manner (i.e., according to Supplemental label) will help future feasibility assessments of rat eradications on islands and the associated levels of risk to non-target species.

Methods

Study Site

Desecheo (18°23′14″N, 67°28′19″W) is a small (1.5 km^2 or 152 ha) island approximately 27 km from the western shore of the main island of Puerto Rico. The terrain is rugged with karst limestone as parent material, and the peak elevation is 218 m. It is dominated by dry forest vegetation, and annual rainfall averages 1020 mm (Seiders et al. 1972). Dryland vegetation includes *Bursera simaruba*-dominated forest, shrubland and grassland. There is no reliable water source on the island (Wetmore 1918). The island is administered by the USFWS as a National Wildlife Refuge. *Rattus rattus* is abundant on Desecheo, and they have apparently been abundant there since first reported in 1912 (Wetmore 1918). Although non-native goats (*Capra hircus*) and non-native rhesus monkeys (*Macaca mulatta*) were once common to the island, they have been recently eradicated (by 2010), or nearly so, with just two individual rhesus monkeys (both female; one of which was sterilized, radio-collared, and released on Desecheo as a Judas) in existence before we began the 2016 operation. Apparently Desecheo had one of the largest nesting colonies of brown boobies (*Sula leucogaster*) prior to rhesus monkeys being introduced to the island. The negative impacts of *Rattus rattus* to natural areas and native species on tropical islands is well known (Shiels 2010; Shiels and Drake 2011; Pender et al. 2013; Shiels et al. 2013; Shiels et al. 2014; **Figure 1**); rats on Desecheo have been observed eating juvenile anoles (*Anolis desechensis*), and scarring on the tails of mature Puerto Rican racers (*Borikenophis portoricensis*) are thought to be caused by rats on Desecheo (Draft EA 2015). Removing rats from Desecheo is expected to restore Desecheo's natural ecosystems by: 1) increasing security for three endemic reptiles (Anole: *Anolis desechensis*, Gecko: *Sphaerodactylus levinsi*, Ameiva: *Ameiva desechensis*), 2) promoting recovery of the island's once abundant seabird breeding colonies, and 3) protecting the federally-listed higo chumbo cactus (*H. portoricensis*).

Rat eradication history and the 2016 bait application Supplemental Label

The first, and unsuccessful, attempt to eradicate invasive black rats from Desecheo National Wildlife Refuge, Desecheo Island, Puerto Rico was made in March 2012 abiding by the main label of USDA APHIS's Brodifacoum-25D: Conservation (EPA Reg. No. 56228-37). This label allows two aerial broadcast applications spaced 5-7 days apart, and application rates up to 18 kg/ha for the first application and 9 kg/ha for the second application. The two aerial broadcast applications were made in compliance with all label directions and use restrictions. The purpose of this eradication effort was to try to help the recolonization and recovery of native wildlife populations on Desecheo Island that have been negatively impacted by the black rat. Several months after the 2012 eradication attempt, rats were observed and deemed survivors rather than new introductions. The operation was subsequently reviewed by external experts (Brown and Tershy 2013), and suggestions were made for improvements, particularly related to planning and operational matters, such as the duration of bait availability in all habitats, to make a future eradication attempt more likely to be successful (Brown and Tershy 2013).

In March/April 2016 (the dry season), USFWS and IC conducted a second eradication attempt on Desecheo Island using Brodifacoum-25D: Conservation (B-25), under a supplemental label specific to this 2016 eradication effort. The concentration of active ingredient for this product based on its label is 0.0025% (25 ppm), and that used for the Desecheo application in 2016 (based on $n = 4$ bulked samples of 10-12 pellets/sample, sampled by NWRC from the batch used on Desecheo April 9, 2016) was (mean ± SE) 0.00272 ± 0.00008% (~27 ppm). Bait was applied under the supplemental label at 30-45 kg/ha (depending upon habitat; see **Figure 2**) for each of two applications (March 18 and April 9) in 2016; these applications rates were 2-3 times greater than those on the main Brodifacoum 25-D: Conservation label used in 2012. Determination of the 2-3 times greater application rate relative to that applied in 2012 resulted from an estimate by experienced personnel with IC. The Environmental Assessment (Draft EA 2015) reported that the bait uptake trial that is recommended prior to determining bait application rates for a rat eradication (see Pott et al. 2015) was the monitoring that was accomplished during the 2012 application where 18 kg/ha was applied during the first application. The 2016 operational goal was to provide a minimum of 30 kg/ha on the ground with allowances for overlapping swaths and additional baiting at zone overlaps and valley bottoms. The label limits each application to the whole island to 5,793 kg. The island is roughly 134 ha (3D surface area), so the theoretical average application rate for the island is 44.2 kg/ha (40.6 kg/ha, or about 4 g/m^2, if bait stations are not included). Each bait pellet used on Desecheo weighed (Mean ± SE) 3.06 ± 0.09 g (n = 49). The label states that the time between application periods is 24 days, but could be 17-66 days depending on weather and/or logistical considerations, and the 2016 applications were compliant with this period between applications (22 days; March 18, 2016 and April 9, 2016), and all other actions were compliant with all label directions and use restrictions.

Monitoring and the role of NWRC

In mid-March 2016, the USDA APHIS WS NWRC (hereafter NWRC) began a monitoring study to evaluate the impacts of the eradication efforts on target and non-target species. Although NWRC directed and supervised this monitoring, field assistants were provided to NWRC by USFWS and/or IC to increase monitoring efficiency and strengthen the interagency partnership. The details of each monitoring component are addressed below, and the summary of this study’s monitoring included the following: 1) Bait application rate (density) monitoring, which entailed counting bait pellets in a series of plots in each of the habitats outlined in **Figure 2**, minutes after each of the two bait applications, 2) Bait availability monitoring, which occurred by revisiting each of the bait application plots at set intervals (e.g., daily for the first week after each bait application), and monitoring bait take by animals by using motion-sensing cameras positioned to observe a subset of bait in plots, and 3) Non-target species monitoring, which entailed a) carcass searches before and after each bait application period, b) assessment of non-target bait take via motion-sensing monitoring cameras, and c) assessment of the levels of brodifacoum residues in focal parts of the food web by sampling (pre- and post-bait application) rats, reptiles, and invertebrates. Based on unpublished findings from the first Desecheo rat eradication attempt in 2012, there were few effects on birds, which, in addition to budget constraints, has justified our general exclusion of targeting birds for residue analyses.

<u>Choice of monitoring site locations</u>

There were 11 sites on Desecheo established for monitoring (**Table 1**; **Figure 2**). These sites were chosen to occupy the different habitats and bait application regions (e.g., deflector, coastal overlap, valleys, cliff; **Figure 2**) in areas accessible (often near established trails) on the western half of the island. Much of Desecheo has steeply sloped terrain and cliffs, and these areas were avoided for safety and logistical concerns. In total, we had four sites in the ‘interior’ (**Figure 2**) that were on ridges or slopes (**Table 1**), two sites in ‘valley floor/bottoms’, one ‘cliff’ site, two sites in the ‘deflector’ zone, and two sites in the ‘coastal overlap’.

At each of the 11 sites, we established a single 160 m transect that had flags marking each 10 m along the transects. The 10 m interval flagging marked the 1 m^2 locations for which we established bait density plots (15 1-m^2 plots per transect; 160 total for each application at all

11 sites). Once in a habitat that had enough area for a 160 m transect, the location of the start of the transect was chosen randomly, and each 1-m^2 plot was systematically marked at 10 m intervals thereafter.

To better describe the habitat at each site, slope and vegetation were described by a single person (Shiels) measuring three variables at each of the 11 sites: average canopy height and maximum canopy height (both estimated by eye to within 5%), and slope (measured with a clinometer). The averages and slopes were integrated (by eye/estimated) across a 20 x 10 m area between stations along each transect, and there were n = 8 of each of these measurements per site.

Below are the detailed methods of each of the monitoring components for this study.

<u>Bait application rate (density) monitoring</u>

On the days of bait application, each of the 11 sites were visited and bait density was measured at each of 15 locations per site. Bait density was measured by placing a 1-m^2 plastic ring (hula hoop) onto the ground at each of the flagged locations along the transect (thus 10 m distance from the next closest plot), and counting the number of bait pellets within the hula hoop (Berentsen et al. 2014). Bait density measurements were conducted as soon after bait was applied as possible, so coordination via radio with the operations team was frequent. If the helicopter applied bait in multiple passes, we would measure bait density after each pass to improve our accuracy of our finalized estimates. Bait density data was made available to USFWS and IC immediately after being recorded, and it was transmitted to the operation team by radio so that time-sensitive adjustments could be made to further maximize efficiency and project success and goals. The same bait density methodology was used in both applications in 2016, and the 1-m^2 plot locations in application 2 were established at random, but within ~2 m and adjacent to, those of application 1.

Bait availability monitoring

The 160 bait density plots were also used for bait availability monitoring to assess the persistence and conditional change of bait pellets after each application. Beginning on the first day of bait application and immediately after counting bait pellets in 1 m^2 plots, two bait pellets were placed side-by-side at the base of a numbered pin flag; all other bait pellets were cleared from the 1 m^2 area. Each day thereafter for one week following each bait application, each of the 15 plots per site were visited to record the number of bait pellets remaining and the condition of the bait pellets according to the Craddock (2004) bait degradation scale, which incorporates details of wet, moldy, damaged, or intact bait; however, due to this index being developed for wet climates, we typically used the general descriptions: 1 = fresh pellets, 2 = cracking, 3 = fracturing/flaking, 4 = pile of dust, and >50% volume bait loss, 5 = unorganized pile of dust, and >75% volume bait loss (**see Figure 3**).

We used motion-activated cameras to monitor a subset of the bait pellets to help determine the types of animals visiting and consuming the bait pellets. Each site always had at least one motion-sensing camera active and monitoring bait pellets, but some sites had two or three monitoring cameras. The cameras were primarily the Reconyx rapid-fire trail cameras, but we also used Browning Model No: BTC-6HD trail cameras. The cameras were set to be triggered by motion, but were also programmed to take a photo each hour (on the hour), and sometimes more frequent at set intervals. Once the Reconyx camera was triggered by motion, it would take 10 consecutive photos over 20 seconds. When monitoring team members would assess bait availability, they would also service the motion camera as needed. For example, if a bait pellet was missing, the monitoring team member would switch out the SD card and view the photos to determine the animal responsible for removing the bait. If both bait pellets were removed from a station with a motion-camera, the camera would be moved to another station within the site where bait pellets were still present.

At the 160 m mark for each transect, we placed two bait pellets into a 30 x 30 x 30 cm cage of ½" hardware cloth to exclude all rats (and other vertebrates) to better understand the longevity of bait pellets in the absence of rats. The bottom of each cage did not have hardware cloth but instead was open to the ground; each cage was held tight to the ground by piling rocks on top of it. The bait pellets in the vertebrate exclusion cages were assessed the same way as the bait availability plots (i.e., baits counted daily and bait condition assessed using the Craddock scale).

Twelve additional 1 x 25 m plots were established by IC to monitor bait availability using the same method used in the 2012 Desecheo rat eradication attempt. Six plots were established at Camp Ridge, which is a grassland site approximately 200 m from Ridge #3; and six plots were established at Long Valley (valley bottom) about 100 m from the edge of Long Valley #1. Once the 1 x 25 m plots were established, and within 2 hours of the helicopter applying bait at the site, 25 bait pellets within each plot were marked with a pin flag and all other bait pellets were removed. Each day after for 1 week, the number of remaining bait pellets were counted and recorded. All bait availability measurements in IC plots and the 11 sites were made available to IC at the end of each monitoring day.

Rat and non-target carcass searches

Carcass searches occurred before, during, and after each bait application period; these involved walking trails to sites, and the transect within each site, while visually scanning the ground for any dead animal (vertebrate and invertebrate, target and non-target) in approximately a 2 m swath on either side of the trail/transect. Equal trail/transects were walked/scanned during each of the carcass searching events (e.g., ‘systematic sampling’); opportunistic carcass search and recovery also occurred outside of the established trails and transects. If any rat or other animal carcass was found, the location, date, and species were recorded and a label with this same information was placed inside a ziplock bag. The carcass was then placed (with gloved hand) into the ziplock bag, and stored cold in a Yedi cooler (on Desecheo), and later in a freezer (on mainland Puerto Rico) until samples could be shipped to NWRC Chemistry lab in Fort Collins, Colorado.

Target and non-target species brodifacoum exposure

Non-target monitoring included: 1) carcass searches before, during, and after bait application (described above), and 2) assessment of the levels of brodifacoum residue in focal parts of the food web by live-sampling animals pre- and post-bait application. We focused our residue sampling on rats, lizards, and insects. We obtained permits from Department of Natural and Environmental Resources (DRNA) in Puerto Rico, and USFWS, for these animal collections. Pre-application residue sampling forms baseline concentrations for which post-application monitoring should exceed if these animals are ingesting (directly or indirectly) brodifacoum, and it also helps establish if there are legacy residues residing in these animals from the 2012 brodifacoum bait applications.

Lizards (ameivas, anoles, and geckos) were hand- or noose-collected, primarily in West and Long Valleys the week before application 1 (n = 20 for each species) and the week after application 2 (n = 13-20 for each species). Once captured, each was euthanized by overdose of isoflurane followed by cervical dislocation; AVMA 2013). Rats (n = 22) were collected the week prior to application 1 with live traps rather than snap-traps because of the abundance of land crabs that may suffer from snap-traps; traps were placed in trees (approximately 2 m aboveground) to limit crab access. All rats were collected in Long and West Valleys. Traps were baited with peanut butter and armed approximately 2 hours before sunset, and checked the following morning within 3 hours after sunrise. Rats were euthanized by overdose of isoflurane followed by cervical dislocation. The sex and weight were determined, and a small (~5 mm length) tissue sample of each rat's tail was placed in buffer solution for DNA preservation and storage at the NWRC genetics lab. Arthropods (mainly insects) were collected by hand or net in Long and West Valleys and Coastal Overlap zones the week prior to application 1 and the week after application 2 (**Figure 2**). Due to the NWRC chemistry lab's protocol requiring at least 2 g of animal tissue to make up one sample, we captured larger-bodied insects (e.g., grasshoppers, katydids, large spiders, cockroaches), and we would also group several individuals of the same type or species into the same sample to reach the 2 g target. All animals live-harvested appeared healthy at the time of collection. Only one bird, a pearly-eyed thrasher (*Margarops fuscatus*), was sampled prior to application 1 after it was found in a rodent trap and euthanized due to injuries it sustained trying to escape the trap. Gloved hands were used when collecting or handling any of these specimens, and all specimens (collected pre- and post-application) were frozen and sent to the chemistry lab at USDA APHIS WS NWRC headquarters, in Fort Collins, Colorado, where they were analyzed for brodifacoum residue (see method below).

Brodifacoum residues in animal tissues were analyzed at USDA NWRC using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method developed for this study. The residue analysis method developed by USDA NWRC is more sensitive than previous HPLC methods (e.g., Pitt et al. 2015), with Detection and Quantitation Limits reduced into the ppb range, rather than ppm range. All residue levels measured above the Detection Limit for the comparable control tissue type were reported, although it should be noted that many of the residue levels for pre-application samples that are reported are below the Quantitation Limits. We also analyzed a subset of all of the samples collected from Desecheo using the HPLC method, which was the method used by this lab in previous studies (e.g., Pitt et al. 2015). Because the HPLC method is less sensitive for detecting low-level brodifacoum residues relative to the mass spectrometer method, we only report the results from the mass spectrometer method.

Results

Bait application rate (density)

Bait application rates that were estimated from the 160 1 m^2 plots that were established across the 11 sites were within the label restriction amounts (**Table 1**). Target application rates were either 30 kg/ha (equivalent to 1 bait pellet per m^2), or 45 kg/ha (equivalent to 1.5 bait pellets per m^2), as each pellet weighed 3.06 ± 0.09 g ($n = 49$). Application 1 appeared to be slightly lighter (average of 1.07 baits pellets per m^2, or 3.3 g/m^2) versus application 2, which had closer to the average desired amount (1.44 baits per m^2, or 4.4 g/m^2). Recall that the theoretical average application rate for the island was estimated at 44.2 kg/ha, yet 40.6 kg/ha, or about 4 g/m^2, if bait stations were not included. Bait stations were established with brodifacoum pellets just after the helicopter application finished on April 9, but they were only established in the three valley bottoms (West, Long, and East Valleys; **Figure 2**). Information from our 160 plots were used by the operations team during the application days to help ensure areas were not missed and to add additional bait in various passes, as needed, by the helicopter. Furthermore, bait densities in application 1 were used to guide application 2; some areas like Long Valley #1 (valley bottom) and the coastal overlap at the foot of Long Valley (Coastal Overlap #1) received a slightly lighter application than desired (0.8-0.9 baits/m^2 measured; 1.5 m^2 was desired), but during application 2 those sites were better covered at the desirable rates (1.2-2.1 baits/m^2 measured) (**Table 1**).

Bait availability

Bait availability differed dramatically among sites (**Figure 4**). Whereas several 1 m^2 monitoring plots in the valley bottoms had both bait pellets removed within minutes after bait application (largely due to hermit crabs, *C. clypeatus*), other plots still had one or both bait pellets present after 20 days (e.g., Coastal overlap #2, grassland). A similar pattern of high variation existed when the two locations containing the 1 x 25 m plots were monitored: some of the plots in Long Valley's valley bottom had all 25 bait pellets removed after 3 nights. In contrast, the grassland 1 x 25 m set of plots at Camp Ridge always had at least 60% of the original 25 pellets for the whole week following bait application (**Figure 4**).

Motion cameras documenting target and non-target species

We estimated there were ~38,000 photos that were taken between application 1 and 2. We reviewed each photo from all 11 sites, and found that there were 2686 photos where an animal was present in the photo. Most of the photos that captured animals showed that they were not in contact with the bait, but instead they were passing by unaware of the bait (e.g., ameiva in **Figure 6**), or perhaps searching or foraging nearby the bait. There were a total of 70 photos from application 1 that showed an animal in contact with a bait pellet; contact was defined as the animal touching the bait with some part of their body, and this included the animal eating the bait and/or removing the bait. The first 5 days following application 1 was the only time that rats were observed in contact with the bait (i.e., March 18-22), and of the 40 photographs involving animals during this period, half of them (i.e., 20) were of individual rats (**Figure 7**). Although rats dominated bait contact and consumption (**Figures 8 & 9**) during the first 5 days following bait application (especially so during the first 2 days), hermit crabs (**Figure 10**) comprised 32% of bait contact and consumption (**Figure 7**). Ameivas, which contacted the bait in 13% of the photographs during the first 5 days, usually had a part of their body (e.g., leg, tail) contact the bait or they touched it with their snout or occasionally were seen giving the bait a single lick and then moving out of the frame. Thus, other than a single lick of the bait, the ameivas were never seen consuming (biting, chewing, swallowing) or removing the bait. Finally, there were two insects (one appeared to be a grasshopper) seen in contact with a bait pellet during the first 5 days following bait application 1 (**Figure 7**).

The last day when a rat was captured by motion-cameras on Desecheo was March 23, which was the sixth day following application 1. On March 23, there was one rat at Coastal Overlap #2 (grass/shrubland at base of West Valley) that was photographed at 6:18pm "searching/sniffing around" the bait pellets, and was 12 cm away from the bait pellets being monitored at its closest point to the pellets. Also on March 23 at 4:06pm, there was one rat on Ridge #2 (forest) that was photographed "passing by" the field of view, and this rat was about 1 m away from the bait pellet at its closest point to the pellet. Therefore, these two rats, which did not contact the available bait when photographed, were the last rats photographed by the motion cameras on Desecheo, despite the cameras being active and bait present in their field of view through April 15, 2016.

There was a total of 30 photos from days 6-20 (March 23-April 7) following application 1 that showed an animal in contact with a bait pellet. Because rats were no longer present or otherwise not photographed by the motion-cameras, the proportion of animals documented contacting the bait shifted (compare **Figure 7** and **Figure 11**), such that hermit crabs comprised nearly half (i.e., 14 of 30) of the photos, and ameivas were photographed contacting the bait in 37% of the photos during this period (**Figure 11**). Insects, primarily grasshoppers, were contacting the bait in four photos, and there was one photo of a black land crab (*Gecarcinus ruricola*) consuming a bait pellet during this time period (**Figure 11 & 12**).

There were obvious differences among sites in the types of animals, and their relative abundances, captured on camera contacting bait pellets. In total, there were only five sites following application 1 that had photos of animals contacting bait, even though all 11 sites had 1 to 3 cameras monitoring bait pellets and all 11 sites had photographs of some animals in the view. For example, the Cliff site only had photos of hermit crabs contacting bait, whereas the Deflector 1 site only had photos of insects (primarily grasshoppers) contacting bait (**Figure 13**). Coastal overlap #2 and Deflector #1 were the only sites that had rats photographed contacting bait, and Long Valley #1 (valley bottom) and Coastal Overlap #2 were the only sites that had ameivas photographed contacting the bait pellets following application 1 (March 18-April 8) (**Figure 13**). It should be noted here that motion cameras were only on a subset of bait at a site, at least monitoring two baits, but in some cases up to six baits at one time; thus, only about 30 baits were being monitored on a daily basis across all sites. We mention this to remind the reader that indeed only a very small fraction of the bait pellets were being monitored with cameras, although continuously, during the post-application period.

Bait pellets were monitored during the first 7 days following bait application 2, which occurred on April 9, 2016. There were approximately 31,000 photos taken and reviewed during this period, and we found that there were 176 photos where an animal was present in a photo. Similar to our findings after the first application, most of the photos that captured animals showed that they were not in contact with the bait. There were 16 incidences where animals were in contact with bait pellets during the week following application 2. There tended to be few proportional changes in animal-bait interactions that occurred from the 6-20 days of monitoring after bait application 1 and the first 7 days of bait application 2. Hermit crabs continued to dominate bait interactions, and insect consumption of the bait had risen to the highest proportional levels of all previous measurements (**Figure 14**). Ameiva interactions tended to decrease after application 2 relative to the latter part of the monitoring period following application 1 (**Figure 14**). There were five incidences of animals contacting bait pellets during days 6 and 7: two hermit crabs, two insects, and 1 ameiva; thus the 0-5 day bait interaction would have been similar to the 0-7 day bait interaction. Furthermore, the photos that captured animals interacting with bait occurred at five of the 11 sites (Cliff, Overlap 1, Ridge 1, Ridge 4, and Long Valley 1) during the week following bait application 2. As with all previous photos, hermit crabs and insects were observed consuming bait pellets, yet ameivas were not seen consuming bait.

<u>Non-target risk: Legacy effects from 2012 rat eradication attempt</u>

Residue analysis of animals sampled the week prior to the first application of the 2016 rat eradication indicated that there were legacy effects of the 2012 rat eradication because brodifacoum residues were present in some animal tissues. Of note, brodifacoum residues were detected in 2 black rats (56.0-58.1 ppb), the 1 pearly-eyed thrasher (134 ppb), 3 Desecheo ground lizards (27.4-41.8 ppb), and 1 cockroach (Order Blattodea; a sample of 18 individuals; 45.5 ppb) during the week prior to the 2016 rat eradication attempt (**Table 2**). These residues findings indicate that brodifacoum residues still remained from the first eradication attempt, 4 years prior in spring 2012, albeit at very low concentrations. It is unknown whether these residues resulted from primary and/or secondary exposure. None of the anoles or geckos sampled (n = 20 for each species) had detectable levels of brodifacoum residue when they were live-harvested the week prior to the 2016 operation; the brodifacoum residues for all target and non-target animals sampled during the week prior to the 2016 eradication can be found in **Table 3**. The detection levels for brodifacoum analysis were: 5.4 ppb for hermit crabs, 7.9 ppb for birds, 8.8 ppb for arthropods other than hermit crabs, 11 ppb for rats and ameivas, and 12 ppb for anoles and geckos.

<u>Non-target risk: Carcass searches</u>

In total, the number of carcasses found post-application of bait were: 22 rats, 10 pearly-eyed thrashers (*Margarops fuscatus*), 1 belted kingfisher (*Megaceryle alcyon*), 1 gray kingbird (*Tyrannus dominicensis*), 1 ameiva, 1 hermit crab, and 1 macaque (**Table 4**). An eel (unknown taxonomy) carcass was also found on a beach, but the sample was lost in transit to the laboratory at USDA APHIS WS NWRC, so residue levels and likely cause of death are unknown. The non-target carcasses that were analyzed for residues included: 7 pearly-eyed thrashers, 1 belted kingfisher, 1 gray kingbird, 1 ameiva, and 1 hermit crab. All rats and pearly-eyed thrasher carcasses found on or after April 7 were too far decayed to collect for further chemical analysis. The bulk of the rat carcasses (found relatively fresh) were from March 22-25 (**Table 4**); however, the monitoring crew that conducted the carcass searches were on Desecheo until March 25, and then returned and searched on March 30-31, and then did not return or conduct carcass searches until April 7. The macaque found dead was the judas (collared, sterilized female), and it was located by following its signal from its GPS collar.

Non-target risk: Brodifacoum residues following the 2016 operation

All residue analysis results for target and non-target animals sampled after the two bait applications in 2016 are shown in **Table 5**. Brodifacoum residues were detected in all rat carcasses sampled post-application. From the non-target animal carcasses or animals live-harvested, the following had detectable brodifacoum residues: 7 pearly-eyed thrashers carcasses (134-2780 ppb), 1 belted kingfisher carcass (2780 ppb), 13 ameivas (the carcass had 844 ppb; the 12 individuals live-harvested had 219-1100 ppb), 13 anoles live-harvested (13.8-233 ppb), 14 dwarf geckos (*Sphaerodactylus levinsi*) live-harvested (12.4-177 ppb), 5 samples composed of multiple understory arthropods live-harvested (10.3-1580 ppb), and 4 hermit crabs (*Coenobita clypeatus*; 3 live-harvested (13.8-24.8 ppb) and the 1 carcass had 36.6 ppb) (**Table 5**).

Discussion

Monitoring efforts associated with the 2016 Desecheo rat eradiation operation uncovered new information about: 1) the variation in bait application rates, as well as the availability and longevity of bait pellets among habitat types, 2) the types and frequencies of species exposed to the bait, and the likely incidences of direct bait consumption versus secondary exposure, 3) the timing of the target and non-target effects when such quantities of bait are broadcasted island-wide, and 4) the persistence and longevity of brodifacoum residues in a dry tropical environment. Below we discuss each of these monitoring findings associated with the 2016 rat eradication operation. The planning and operational improvements that were suggested by Brown and Tershy (2013) after the 2012 Desecheo rat eradication attempt appeared to be followed for the 2016 attempt, and live rats or their signs of survival have not been observed since March 23, 2016 (i.e., 6 days following bait application 1). Furthermore, assessments by USFWS and IC in November 2016 and April 2017 have resulted in the conclusion that Desecheo is now rat-free (announced in June 2017).

Bait application rate (density)

Brodifacoum 25D: Conservation bait was generally applied over Desecheo at the targeted rate, and the maximum amount of bait per application of 5,793 kg was not exceeded. It is common for these types of operations to have slight discrepancies among the different types of methods for which bait application rates are measured; in particular, the amount of bait added to (and exiting from) the bait-hopper on the helicopter does not always match what is measured on the ground. There are several factors that may account for this, and one being that ground monitoring is always constrained by affordable time and effort—thus, all bait pellets cannot be recovered or measured across the whole island and the number of plots that is realistic for such time-sensitive measurements is constrained. Much of Desecheo is inaccessible due to safety concerns (e.g., steep slopes and cliffs), and the monitoring team was restricted to areas that could be accessed by foot (walking) in a reasonable time. Because the helicopter often had to conduct multiple passes (usually two) at areas receiving 45 kg/ha, our monitoring team would make bait density measurements following each pass. Measuring bait density after each pass certainly improved the accuracy of our bait density estimates shown in **Table 1**; however, each set of 15 plot measurements at a site generally took 15-45 minutes (depending on whether 1 or 2 personnel were measuring, and also on the ground vegetation and terrain; **Table 1**). Another logistical aspect that may have influenced the accuracy of our bait density measurements was the delay from when bait was applied by the helicopter to when the monitoring team was given the go-ahead to begin measuring bait density on the ground. During bait application 1, it was typically 40-90 minutes from when bait was deposited onto the ground at a given site to when bait density measurements began at the site; however, we were able to shrink this gap from bait application to beginning bait density measurements to 15-40 minutes for application 2. Furthermore, we noticed hermit crabs removing bait within a few minutes of bait falling to the ground, which probably influenced the accuracy of our bait density estimates. A final possibility explaining why some sites had lower bait density than our target densities (or that measured exiting the hopper) may have been due to some bait pellets being retained in trees, thereby affecting the densities that we were assessing on the ground. In general, bait appeared to fall easily through tree branches at the sites that had trees, but it is possible that some of the large-diameter trees like *B. simaruba* may have retained some bait pellets. Although some of these logistical concerns may be able to be improved for future operations of this kind, perhaps the most helpful aspect of having bait application rates measured on the ground is to provide 'real time' data to help inform the operations team during the applications so that adjustments can be made to help ensure that the target bait applications are met in all habitat types.

Bait availability

High variation in bait availability throughout our study was expected due to the pronounced differences in habitats sampled (e.g., forest, grassland, shrubland, rocky and sandy coastline; **Table 1**), and the associated wide ranges in abundance and activity of animal life. The valley bottoms, which contained substantial leaf litter and tree canopy cover, had the most rats and hermit crabs of all habitats on Desecheo (Draft EA 2015; personal observation). It was within the valley bottoms, and to a lesser extent some ridge/slope environments, where we observed hermit crabs removing bait pellets within minutes of the pellets contacting the ground; the valley bottoms also had the most rapid measured losses of bait pellets (**Figure 4**). The 1 x 25 m plots set up by IC allows for a direct comparison between the 2012 and 2016 applications because the same locations (Long Valley's valley bottom, and Camp Ridge's grassland), number of plots (12 total, each 1 x 25 m), and methodology were used in both years. On average across all 12 plots, 51% of the bait was consumed (note: for these purposes, any bait missing was assumed to have been consumed) within the first three nights in 2016, whereas 60% of the bait was consumed within the first three nights across the 12 plots in 2012 (Draft EA 2015). Bait consumption differed between habitats in both years; however, there appeared to be slightly more variation between the two sites in 2016 than in 2012. In 2012 in the grassland, an average of 50% bait was consumed within the first four nights, whereas in the woodland habitat (valley bottom) about 60% bait was consumed (Draft EA 2015). In 2016, on average, just 28% of bait was consumed within the first four nights in the grassland, and an average of 73% of bait was consumed within the first four nights in the valley bottom (**Figure 4**). Although these two sets of measurements occurred in the same areas and the same time of year (March), there are many factors that can influence bait take at a site. However, one explanation for the apparent difference between sites in 2012 and 2016 may be due to the spatial bottleneck of rats from the 2012 eradication attempt; surviving individuals would have probably colonized the most desirable habitats first (i.e., woodland/forest in valley bottoms). Additionally, there appeared to be less rats on Desecheo in early March of 2016 relative to 2012 (IC, personal communication).

With the reduction and possible island-wide elimination of rats resulting from the first bait application in 2016, there were fewer bait consumers present from application 1 to application 2; thus, the bait persisted in the environment for a greater period following the second application than after the first application. Bait removal/consumption was slower after the second application relative to the first, and this pattern holds true for both the 2012 and 2016 operations. In 2012, an average of 39% of the bait remained on the ground after 10 nights following application 2 (based on 12 IC plots, each 1 x 25 m; Draft EA 2015); however, the valley bottoms, and otherwise areas with high abundances of hermit crabs, continued to have substantially high bait removal. In fact, 90% of the bait pellets were consumed in the valley bottom within the first five nights after the second application in 2012, and all bait pellets had been consumed within 8 nights. In contrast, about 59% of the bait pellets remained on the ground after 10 days in the grassland habitat after application 2 (Draft EA 2015). Just prior to the second bait application in 2016, which was 20 days since application 1 and 14 days since a rat had been observed alive on Desecheo, there were bait pellets remaining in what appeared to be palatable form (e.g., 2 or 3 on Craddock scale; **Figure 3**) in several habitat types, but there were no remaining bait pellets found in West or Long Valleys. The decisions regarding how much bait to be used during each application is a delicate balance of ensuring there is excess such that all rats have bait in their nightly home-ranges for at least 4 nights, but also minimizing how much excess bait is added to the island that rats are not consuming. Thus, from a practical point of view, and one that is focused on ensuring rat eradication success during such $1 million+ operations, there is no set, standard, or blanket amount of bait that should be applied to any island to ensure success. The rodenticide product label states the maximum allowance, but the amount that is needed could be more than allowed on the main label and therefore require a supplemental label specific to that island. The bait application amount should reflect on-island bait availability trials just prior to embarking on a bait application operation, the on-island monitoring feedback (i.e., bait availability monitoring and trail camera results) occurring during and between application periods, as well as other associated on-island ecological research that can help hone the appropriate amount of bait needed to ensure a high probability of success (see Pott et al. 2015 for further discussion).

Target and non-target bait take

The diversity of animals that were documented contacting the bait included several insects and four other animal species (rat, hermit crab, black crab, ameiva). During the first five days following application 1, 82% of the photos of animals contacting bait were either rats (50%) or hermit crabs (32%). The animals contacting bait as documented by the motion-cameras were not equally represented across sites (see **Figure 13**), and during the first 5 days since application 1, the baits monitored at Cliff and Long Valley#1 (bottom of valley) were mostly contacted by hermit crabs, whereas rats were in common contact with the bait at Ridge #4 and Coastal Overlap #2, and the photos of the Deflector baits were only visited by insects. It was surprising that there were no photos of rats contacting bait in Long Valley#1 during the first 5 days after bait application, but there were only two cameras functioning and monitoring baits (4 baits in total) during this period and these results also underscore the prevalence and importance of hermit crabs as bait competitors at sites where rats were also abundant. Also of interest and surprise was that Long Valley #1 had no hermit crabs observed in contact with the bait during days 6-20; one possible explanation for this was that the hermit crabs were satiated from 5 days of feeding on bait pellets, and they possibly hoarded bait pellets at their burrows in Long Valley. Coincidentally with less visitation and contact to monitored baits by hermit crabs during the 6-20 days following application 1, there was an increase in the proportion of bait contact with ameivas at Long Valley #1 and Coastal Overlap #2 (**Figure 13**). Although ameivas were never documented having bait pellets in their mouths or otherwise feeding on the bait pellets, their increased proportional interaction with the bait during the 6-20 day post application period may have been due to less competition for arthropod prey with reduced activity from rats and hermit crabs, many bait pellets remaining in their regular foraging microhabitats (i.e., ground and leaf litter), or possibly ameivas being more attracted to the remaining bait pellets because insects (i.e., prey for ameiva) may have been frequently visiting bait pellets.

Motion cameras did not show any evidence of birds contacting the bait or in the camera's field of view around the bait. The most common land bird on Desecheo is the pearly-eyed thrasher, and the population has apparently persisted for the past 100 years since it was identified as the only passerine during one of the first recorded bird censuses on Desecheo (Wetmore 1918). Results from carcass searches and brodifacoum residue analyses showed that pearly-eyed thrashers were exposed to brodifacoum in 2016 (either directly or indirectly), and some individuals likely died from such exposure (see below). Because motion camera showed no evidence of direct bait consumption, pearly-eyed thrashers that died after bait application were most-likely secondarily exposed to brodifacoum, probably through consumption of brodifacoum-containing insects, other arthropods, and possibly small lizards like anoles and geckos. Pearly-eyed thrasher diets in Puerto Rico have been recorded as being mostly fruit and other vegetative material (87%), yet animal comprises approximately 13% (Wetmore 1916). Furthermore, anoles are frequently preyed upon by pearly-eyed thrashers in several islands in the northeastern Caribbean (McLaughlin and Roughgarden 1989), and the majority of anoles that we sampled contained brodifacoum.

Non-target risk: Carcass searches

Aside from the 22 rat carcasses found, there were 16 non-target carcasses found (12 birds, 1 ameiva, 1 eel, 1 hermit crab, and 1 macaque) following the 2016 Desecheo Brodifacoum 25-D: Conservation applications. The bulk of the rat carcasses (found relatively fresh) were from March 22-25; however, the monitoring crew that conducted the carcass searches were on Desecheo until March 25, and then returned and searched on March 30-31, and then did not return or conduct carcass searches until April 7. This means that rats began to die (showing up in carcass searches) within 4 days of the first application of bait (4 dead rats were found). The peak in dead rat carcasses was 5 days after the first application (on March 23) when 9 dead rats were found; on this same day, the belted kingfisher was also found dead. The kingfisher had elevated levels of brodifacoum, and most likely died from brodifacoum exposure, yet it is unknown if this fish-preying bird died from direct exposure (i.e., eating the bait pellets) or by eating other organisms like fish that were exposed to brodifacoum during the previous days. The dead eel (unknown taxonomy) carcass was also found on a beach, but the sample was lost in transit to the laboratory at USDA APHIS WS NWRC, so residue levels and likely cause of death are unknown. The eel and kingfisher were the two non-target mortalities that were most-likely exposed to brodifacoum toxicant in the near-shore environment.

The death of the rhesus macaque was also surprising, especially because a larger troop of macaques survived the 2012 rat eradication attempt that also used brodifacoum bait pellets applied through aerial broadcast. The rhesus macaque that was found dead on April 9, which was 22 days after the first application, and it was located by IC staff following the signal from its GPS collar. The macaque was found on the shoreline on the north side of the helicopter pad, and the IC staff member who found it estimated that it had been dead for about 2 weeks. Rhesus macaques were introduced to Desecheo Island by the U.S. Department of Health, Education and Welfare's National Institutes of Health in 1966 as part of a primate ecology project that was terminated in 1971. The remaining population of rhesus macaques was largely eradicated in the late 2000s by IC, USDA APHIS WS, and USFWS to promote the recovery of nesting seabirds and native plants and reptiles. Two individual macaques had evaded removal efforts, and were present on the island when the 2016 rat eradication began. Due to potential risk of exposure to B-virus (*Cercopithecine herpesvirus*) and other potential zoonoses, the rhesus macaque carcass found on April 9 was not sampled. Therefore, no residue data is available for this animal and the cause of death is unknown; however, in all likelihood, and given the coincidental timing of the operation and the macaque's death, this individual probably died from consumption of the brodifacoum bait pellets or by consuming animal prey or carcasses that contained brodifacoum. The death of this individual was somewhat surprising because there were more macaques present on island during the 2012 brodifacoum application, and evidence from GPS collars and trail cameras revealed that the small population survived the 2012 operation. Many factors, including drier conditions and/or less available resources in 2016 vs. 2012, and the possibility of some legacy levels of brodifacoum in the macaques prior from 2012, may be some of the possibilities for the macaque mortality in April 2016. The final macaque individual (non-sterilized and noncollared female) remaining on Desecheo has been recently confirmed with trail cameras (S. Silander, personal communication in July 2017); though macaques are now considered functionally extinct on Desecheo.

Non-target risk: Legacy effects in 2016 from the 2012 rat eradication attempt

Our sampling the week prior to the 2016 rat eradication attempt revealed that brodifacoum residues can persist in parts of the food web for up to 4 years. The longest previous documentation that we are aware of that shows brodifacoum persistence in the biological environment was 2.1 years in lava lizards (*Microlophus duncanensis*) in the Galápagos Islands (Rueda et al. 2016). Although we expected some ameivas may have contained residues from the 2012 brodifacoum application when we live-harvested the week prior to the 2016 brodifacoum application, we did not expect any other animals to show the legacy effects that we found. In addition to the three ameivas, there were two rats, one pearly-eyed thrasher, and one cockroach sample (18 individuals) containing detectable levels of brodifacoum (27-134 ppb; **Table 2**). Ameivas are estimated to live 3-6 years on Caribbean Islands (N. Angeli, personal communication), and therefore some individual that were sampled for brodifacoum before the bait application in 2016 could have been alive, and exposed to brodifacoum, during the 2012 operation. Survival after exposure to the 2012 brodifacoum applications remains as one possible explanation for the presence of brodifacoum residues in liver tissues of three individual ameivas sampled just prior to the 2016 operation. Similarly, the pearly-eyed thrasher, which we had not intended to harvest prior to the 2016 operation, could have been present on the island in 2012 and 2016 as the average life span for pearly-eyed thrashers in dry forest is 5.5 years (median: 5 years, mode: 3 years; Arendt 2006). However, this hypothesis that 2012 surviving individuals were sampled in 2016 would not likely apply to the other two species with legacy brodifacoum in their tissue because they live <4 years in the wild. Specifically, rats and cockroaches are typically estimated to live <1 year in the wild. Through mark-and-recapture sampling in Hawaiian mesic forest, Shiels (2010) discovered that the maximum longevity of black rats (both sexes) was about 19 months. In New Zealand forest, the maximum longevity recorded for black rats was 11 months for males and 17 months for females (Daniel 1972). In the laboratory, mean longevity for black rats can be 3.9 years for males and 3.4 years for females (Bentley and Taylor 1965). The American cockroach (*Periplaneta americana*), which is a different species than the much smaller, brown-colored, cockroaches sampled in our study, has a life span of about 2 years as an adult (Willis and Lewis 1957). A second hypothesis to explain the legacy brodifacoum in animal tissue from the 2012 bait application is that the very hydrophobic brodifacoum compound breaks down in the soil slowly, and as it is retained there for several years such that animals that spend a portion of their life cycle on or in the upper soil layer (e.g., insects) would gain direct exposure that could then be transferred up the food chain during the period from March 2012 to when the last pre-application animal was live-harvested on March 17, 2016. Furthermore, it is unknown how invertebrate's metabolic processing affects the brodifacoum molecules once bait consumption takes place. As hermit crabs and other invertebrates are not affected by the anticoagulant brodifacoum (i.e., they may eat it and not die or become sick), it is possible that they may be recycling the majority of the brodifacoum through their bodies—bait pellet consumption to excrement—and onto the soil surface. It is unclear if this truly represents brodifacoum cycling in invertebrates, and there was an absence of available literature covering this topic when this report was written, but it does seem like this pathway deserves more investigation, especially when terrestrial crabs are consuming (on Desecheo) an estimated onethird of the brodifacoum bait or more, and insects are a common prey item for many predator species (e.g., lizards, birds, mammals). Other islands like Palmyra experience greater land crab densities than Desecheo, again making the understanding of the brodifacoum cycling in these non-target invertebrates particularly important for future island eradications using brodifacoum.

Although brodifacoum was present in the six vertebrates and one insect sample 4 years after any use of brodifacoum occurred on Desecheo, the brodifacoum levels were low (27-134 ppb). These low levels were only detectable on the mass spectrometer method, and not the HPLC method when a subset of the same samples were analyzed using both methods (D. Goldade, unpublished data). However, for the brodifacoum residue analysis on Palymra that was similar to Desecheo's but used the HPLC method only, the levels of detection indicate that detection should have been possible for six of the seven Desecheo samples (i.e., not the 27 ppb sample) that had legacy residues because the detection level for the Palmyra samples was 11-29 ppb; Pitt et al. 2015). The detection level used in Rueda et al. (2016) for the Galápagos study with lava lizards was 10 ppb, and all of the liver samples from lava lizards about 2.1 years after brodifacoum application were <200 ppb and several were just above the detection limit. Although the aforementioned Galápagos and Desecheo brodifacoum levels are quite low, there is still concern for accumulation of brodifacoum in predators of these brodifacoum-exposed prey (lizards, birds, and insects) over time because of the persistent nature of the brodifacoum molecule that we have found on Desecheo and that Rueda et al. (2016) has found in the Galápagos. Moreover, Rueda et al. (2016) found a dead short-eared owl (*Asio flammeus*) 773 days (2.1 year) post-baiting that had 577 ppb brodifacoum in the liver, indicating that predator consumption of prey containing brodifacoum residue can result in mortality years after a brodifacoum bait application to eradicate rats from a tropical island. Thus, some concern of delayed mortality from brodifacoum poisoning may be warranted for Desecheo's birds of prey. A pair of kestrel were observed before, during, and the few weeks after the Desecheo bait application of 2016. And, although the 10 pearly-eyed thrasher carcasses found post-bait application most likely died from brodifacoum exposure, there was not a noticeable change in the resident pearly-eyed thrasher population on Desecheo. Kestrels and pearly-eyed thrashers are known to prey upon anoles in the Caribbean (Wetmore 1916), and this was also noted during field observations on Desecheo during the 2016 rat eradication. Therefore, these birds of prey, and possibly others such as red-tailed hawk, osprey, and peregrine falcon that are not likely residents of Desecheo but were seen flying and hunting on Desecheo in 2016, are species that are strong candidate for long-term brodifacoum exposure and possibly delayed mortality from consumption of brodifacoum-exposed prey.

<u>Non-target risk: brodifacoum residues post-application in 2016</u>

Studies of more immediate (days to weeks) non-target exposure to brodifacoum through tissue residue analysis have been more common than long-term (years) studies. On Desecheo, brodifacoum was well integrated into the surviving food web as tissue residues were found in about 20% of the sampled insects, 100% of the sampled hermit crabs, and 75% of the sampled lizard community (**Table 5 & 6**). Residues in non-target carcasses also indicated that brodifacoum likely entered the near-shore marine environment and land bird community. We expected that brodifacoum would be widely integrated into the food web from this rat eradication operation, as this has been repeatedly demonstrated in previous rat eradications that have used brodifacoum (e.g., Dowding et al. 1999; Masuda et al. 2014; Masuda et al. 2015; Pitt et al. 2015).

Brodifacoum exposure to the lizard community was of particular interest as we planned for the Desecheo rat eradication because there is little information about brodifacoum's primary and secondary poisoning of lizards (Hoare and Hare 2006; Harper et al. 2011). Harper et al. (2011) found six Galápagos land iguanas (*Conolophus subcristatus*; mean adult length is 100 cm) had died within 2 months after a rat eradication of Isla Seymour Norte (184 ha island within the Galápagos Islands) when a much lower bait application rate was used (3 kg/ha) than on Desecheo, but the bait had double the brodifacoum concentration (50 ppb instead of 25 ppb) as used on Desecheo. The six land iguanas apparently died from brodifacoum toxicity, and they represent approximately 4.5% of the total Galápagos land iguana population on the small island rat eradication. While the number of ameiva on Desecheo is unknown, the single mortality, which is uncertain if its death resulted from brodifacoum, that was found approximately 5 weeks after the first bait application certainly represent < 1% of the island's population of ameiva. Furthermore, during an intensive mark-and-recapture study on Desecheo where 57 ameiva and 453 anoles were marked prior to the 2012 bait application, there was no significant change in short-term survival or population-level impacts due to the brodifacoum baiting (J.L. Herrera-Giraldo et al., unpublished data). As Harper et al. (2011) concluded from their study in the Galápagos after findings Galápagos iguana mortalities 2 months after bait application, extended monitoring of lizard populations is probably warranted.

There was no evidence that ameivas directly consumed bait pellets in the Desecheo rat eradication attempt; motion-cameras only showed a single lick of the bait pellet or a touch with their snout or other body parts. Similarly, during a feeding trial with Galápagos land iguanas, Harper et al. (2011) observed that none of the test animals accepted/consumed any brodifacoum bait. Furthermore, when Witmer and Mauldin (2012) conducted risk assessments via captive feeding trials using ameivas from South America, they gavaged the test animals to ensure brodifacoum or diphacinone exposure. Of the 14 ameivas gavaged with brodifacoum (six individuals at 1x concentration, and eight individuals at 10x concentration), a total of four ameivas died. Although many tropical lizards are omnivores, the field observations on Desecheo Island and current stomach content analyses at NWRC of captured individuals used for residue analysis indicate that ameiva probably do not consume the brodifacoum bait directly but instead gain exposure to the residues through litter-arthropod (mainly insect) ingestion.

Ameivas, anoles, and geckos (all being insectivores) apparently tolerate some levels of brodifacoum residues, at least during the period that we measured them, which was approximately 4 weeks after first bait application. During this period, ~60% of the post-application geckos and anoles had brodifacoum residues (~10-20 ppb) when live-harvested, whereas all of the live-captured ameivas had brodifacoum residues (~200-800 ppb). The brodifacoum LD50s for these lizard species are unknown. Although Witmer and Mauldin (2012) orally gavaged ameivas with brodifacoum, and three ameivas died with 100 ppb brodifacoum residue levels at the time of death, it is uncertain if brodifacoum was the cause of their death; therefore the residue levels of non-surviving individuals from their study should not be used as an estimated lethal concentration. Instead, the 800 ppb brodifacoum residues of the only field-collected ameiva carcass on Desecheo may give some indication of the level of brodifacoum in tissue that would indicate toxicosis from brodifacoum.

Brodifacoum LD50s for other non-targets species (e.g., birds, hermit crabs, insects, spiders) that we sampled on Desecheo are also unknown. However, Dowding et al. (1999) reported that 500 ppb is the lethal threshold for a species of New Zealand bird. If these values were to be applied to non-target vertebrate species in our study, then all the pearly-eyed thrashers, ameiva, and belted kingfisher carcasses would be well above the lethal threshold, indicating their most likely cause of death was indeed from brodifacoum poisoning. The single hermit crab found dead after bait application contained brodifacoum residue in its tissue, but it is unlikely that brodifacoum was the cause of its death given that invertebrates are not known to be affected by anticoagulants such as brodifacoum. To further help inform us of the levels of brodifacoum that may be lethal for both target and non-target animals sampled, tissue residue concentrations are listed in **Table 6** for animals found dead and live-harvested following Desecheo's 2016 rat eradication and Palmyra Atoll's 2011 rat eradication.

Biological cost-benefit considerations of rat eradications using brodifacoum

Two islands in Haida Gwaii, British Columbia, Canada, were recently declared rat-free after aerially delivering brodifacoum rodenticide—this was Canada's first eradication of black rats using an aerially broadcasted rodenticide (http://coastalconservation.ca/projects/night-birds-returning-haida-gwaii-british-columbia/). Like all rodent eradications from island that use toxicants as the method to eliminate the target species, non-target species are also exposed to the toxicant (Masuda et al. 2014; Masuda et al. 2015; Pitt et al. 2015; Croll et al. 2016). In the case of Murchison and Faraday Islands in Haida Gwaii, the rat eradication using aerially delivered brodifacoum bait resulted in 120 non-target animals killed by the poison; most of which were crows and ravens, but carcasses of sparrows and a thrush species were also found following this rat-eradication effort (Zeidler 2016). In the tropical Pacific (Palmyra Atoll), carcasses of 84 non-target animals, representing 15 species of birds, fish, reptiles, and invertebrates, were found dead with brodifacoum residues in their tissues after a black rat eradication using at least three times the amount of brodifacoum bait as used on Desecheo in 2016 (Pitt et al. 2015). The non-target mortalities and brodifacoum levels in target and non-target species are contrasted between Desecheo and Palmyra in **Table 6**. Additionally, the infamous Rat Island (Hawadax Island, Aleutian Archipelago, Alaska) Norway rat (*R. norvegicus*) eradication using brodifacoum applied aerially resulted in the death of 22 bald eagles (*Haliaeetus leucocephalus*; Croll et al. 2016). Despite these significant and immediate non-target species mortalities documented on Rat Island, Palmyra, Murchison and Faraday, during rat eradications using brodifacoum, the documented increases in native flora and fauna also have been significant, and generally show that the relatively few non-target mortalities are far outnumbered by the increases in nest success, adult populations of rare species or species that had not been documented when rats were present, and plants (Croll et al. 2016; Zeidler 2016; A. Wegmann, unpublished data). Although it is critical to plan and implement strategies to limit risk to non-target species exposure and mortality to toxicants prior to and during rat eradication operations, it is equally critical that all parties involved in invasive rodent eradications involving anticoagulants such as brodifacoum baits understand that non-target mortality is inevitable. Thus, the decision that land managers must make is whether the biological costs (i.e., non-target species exposure and mortality) outweigh the biological benefits (i.e., increased native populations, particularly of missing or reduced species that were present prior to rodent establishment).

Key Conclusions to Inform Management for Desecheo and Other Islands

We highlight some of the key results and conclusions from our study in effort to apply these toward improved management of invasive species on Desecheo Island and other similar islands. Our highlighted results also associate with the Desecheo National Wildlife Refuge Comprehensive Conservation Plan (Silander et al. 2012), outlining the subsequent 15 years of conservation planning for the island. Continued efforts to link rat removal with native species responses in a continuously rat-free island will serve as a model for local, regional, and international interest groups (public, land managers, conservation biologists, funding agencies), and it will hopefully apply to future islands that currently suffer from establishment of non-native rodents.

- Brodifacoum has long been proven to be an effective poison for use in rat eradications on islands. Interest groups are often concerned about the use of this toxicant relative to others that are less potent/toxic (e.g., diphacinone). In the case of Desecheo, brodifacoum indeed appeared to be the better choice of toxic baits given its proven effectiveness in eliminating *R. rattus* on hundreds of islands worldwide, and because the risk to non-target species was relatively low—in particular, there were 1) very few birds on the island (seabirds, forest birds, and raptors), 2) the native/endemic fauna is dominated by reptiles that appear to be at little risk (due to an absence of their direct bait consumption, and high survival during use of brodifacoum in 2012 and in other studies) and invertebrates that are not known to be negatively affected by the anticoagulant, and 3) no humans, livestock, or pets reside on the island. Successful rat eradications have occurred with alternative toxicants to brodifacoum (see Witmer et al. 2007; Witmer et al. 2011), and these should be considered in the future, especially when risks to non-targets are higher than they were on Desecheo.
- Biological sampling the week prior to the 2016 rat eradication attempt revealed that brodifacoum residues can persist in parts of the food web for up to 4 years. This was unexpected and there are no other studies that we know of that have monitored for brodifacoum in the food web for such an extensive period following bait application.

That being said, the concentrations in the captured animals were quite low (27-134 ppb), and because of this the risks posed to animals on Desecheo after 4 years post-application appear to be small. However, our findings highlight a much greater concern if this rodenticide is applied more frequently to an island or fragment of an island, such as commonly practiced during rat control projects. In fact, second generation anticoagulants (mainly brodifacoum and bromadiolone) have been the poison type most used in rat control efforts on islands worldwide (Duron et al. 2017). Finally, there can be delayed mortality (e.g., short-eared owl, 2.1 years after island-wide brodifacoum application; Rueda et al. 2016), particularly in raptors, when feeding occurs on prey such as lizards and insects that we now know may retain brodifacoum for up to 4 years. Therefore, long-term monitoring of raptors, and other birds of prey (like pearly-eyed thrashers), would be of interest.

- NWRC scientists were impressed with the professionalism and level of detail practiced during the 2016 bait application on Desecheo; certainly this was a reflection of IC's years of experience conducting such operations, as well as its highly qualified staff and partners. Although many of the standard operational and monitoring protocols used in this Desecheo study are common to IC's protocols (e.g., Witmer et al. 2007; Keitt et al. 2015), we have a few minor recommendations that may benefit future studies and operations of this kind. We recommend the use of motion-cameras positioned to monitor individual bait pellets—this gives an indication of, and ability to quantify, the types of animals visiting and consuming/removing the bait, and the time since bait application when these activities occur. Second, we recommend the use of the hula hoop method for bait density measurements (Berentsen et al. 2014), and the subsequent monitoring of individual baits next to pin-flags. The hula hoop method is quick and easy, allowing for much of the island to be covered with little setup or required personnel; and it allows for a high number of replicate plots. Finally, as much as possible on islands like Desecheo where there are diurnally active crabs or other non-targets that consume bait, it is recommended that the bait application occurs late in the day to maximize the bait available to rats, which are generally most active at night. We estimated that at least 10% of the bait in valley bottoms was removed/consumed by hermit crabs during the several hours before nightfall on Desecheo. Moreover, we also suggest future research into developing a crab deterrent that would be incorporated into the rodenticide bait.

- With the hopeful absence of goats, monkeys, and now rats, there are two other invasive animals on Desecheo that are concerning: green iguana (*Iguana iguana*), and the red imported fire ant (*Solenopsis invicta*). The fire ant species was confirmed in October 2016 as *S. invicta* using molecular techniques similar to those described in Shiels et al. (2017) by Dr. Will Haines, University of Hawaii. As both of these invasive animals pose threat to Desecheo's native flora and fauna, investigations into their local impacts, control/suppression, eradication, and biosecurity should be pursued as soon as possible.
- Given the relatively recent (<8 years) eradication of goats, macaques, and now rats, it complicates one's ability to confidently attribute the near-future changes that are anticipated in the native plant and animal populations on Desecheo to rat removal (even more so now with green iguana established). From an island-wide restoration perspective, it may not matter which mammalian removal(s) account for specific changes in Desecheo's ecosystem; however, biological monitoring post-application is certainly warranted, and knowing what populations rats were suppressing can be helpful for

planning and prioritizing other island removals of invasive rats. Although one biological response may have already been present from rat suppression (the native butterfly, *Eunica monima*; Shiels et al. 2017), we recommend monitoring efforts for Desecheo that focus on rat-vulnerable species, or the likely candidate species that have rat-vulnerable characteristics (e.g., small-egged birds, Zarzoso-Lacoste et al. 2011; large-bodied, slow-moving, or nocturnal insects, St Clair 2011; large-seeded (>2 mm length) plants, Shiels 2011; Shiels and Drake 2011; Pender et al. 2013).

- Biosecurity will now be a significant issue for keeping Desecheo rat free. Surveillance, using multiple techniques, perhaps including traps and counters on automatic traps, trail cameras, chew tabs, tracking tunnels, sniffer dogs, eDNA technology (NWRC has a lab specializing in this), FLIR night technology, drones, and frequent visitation by a trained team looking for signs of rats, are recommended. Hopefully a rapid response plan is, or will be shortly, in place so that trained personnel that are already on payroll or in contract will be able to respond to rat detection before individuals become established.

Acknowledgements

It was clear that all people involved in this multi-agency partnership (USFWS, IC, USDA, DNER, NZ pilot, AK mechanic) had great care in doing their part to make this rat eradication effort successful, as well as learn as much as we could from this large effort. We are grateful for the many people involved in this study, ranging from those on the Desecheo steering committee, operations, monitoring, product registration, DRNA and USFWS for permits, on-island law enforcement and safety, chemical analysis, photo and data analysis, and logistics. Special recognition goes to Kirsty Swinnerton (IC), Cielo Figuerola-Hernández (IC), David Will

(IC), and Susan Silander (USFWS) for doing the majority of the planning, organization, and execution of the project. The core monitoring crew members included Kirsty Swinnerton, Cielo Figuerola-Hernández, Ricardo Colón, Juan García-Cancel, Jorge Gutiemez, Armando, Claudia Lombard, and Erick Bermudez. Jose Luis Herrera-Giraldo, Juan Carlos, Pablo, Erick Bermudez, and Nicole Angeli provided critical assistance with lizard capture/sampling. Familiar faces in IC's heli-ops included Jason, Tommy, and Richard. Special thanks to the chefs (Izzy and Hulk), and to Jose Rojas with USDA APHIS VS who stored and shipped the frozen animal samples.

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Table 1. Bait application rates (mean +/- SE bait pellets per m^2) and ground cover vegetation (0-1 m height) measured on the ground in 1 m^2 plots (n = 15 for each site) along 150 m transects in Desecheo Island, Puerto Rico. Target application rates were either 30 kg/ha (equivalent to 1 bait pellet per m^2), or 45 kg/ha (equivalent to 1.5 bait pellets per m^2, and listed in **bold**), as each pellet weighed 3.06 ± 0.09 g (n = 49). Vegetation ground cover values were from Application 1.

Site	Habitat description	Average & (Maximum) Canopy Height (m)	Slope (%)	Vegetation ground cover (%)	Application 1 (Bait pellets/m^2) (March 18, 2016)	Application 2 (Bait pellets/m^2) (April 9, 2016)
Deflector #1 (coastline of Long Valley [L.V.])	Coastal; rocky with herb/grass	0.2 ± 0.1 (2.5 ± 0.4)	2.4 ± 0.8	62.0 ± 7.3	1.6 ± 0.4	1.6 ± 0.3
Deflector #2 (coastline of West Valley [W.V.])	Coastal; sand with little to no vegetation	0.1 ± 0.0 (0.7 ± 0.3)	4.4 ± 2.2	3.3 ± 3.3	0.6 ± 0.2	1.6 ± 0.4
Coastal Overlap #1 (50-80 m inland of high tide line, L.V.)	Mixed shrubland with herbs, grass, small trees	1.3 ± 0.2 (4.0 ± 0.1)	7.3 ± 1.5	15.3 ± 3.9	**0.9 ± 0.2**	**1.2 ± 0.3**

Coastal Overlap #2 (50-80 m inland of high tide line, W.V.)	Thick grassland and scattered shrubs	0.7 ± 0.1 (3.0 ± 0.2)	4.4 ± 1.5	75.0 ± 8.0	**1.8 ± 0.2**	**0.7 ± 0.3**
Valley Bottom #1 (L.V.)	Forest	3.3 ± 0.1 (7.0 ± 1.1)	15.4 ± 1.9	25.0 ± 4.8	**0.8 ± 0.2**	**2.1± 0.4**
Valley Bottom #2 (W.V.)	Forest	3.5 ± 0.2 (9.3 ± 0.7)	18.4 ± 2.4	20.8 ± 4.7	**1.4 ± 0.3**	**2.1 ± 0.4**
Ridge/Slope #1 (West Ridge of W.V.)	Forest edge and open shrubland	2.6 ± 0.3 (6.9 ± 0.7)	10.4 ± 2.4	10.2 ± 2.4	1.2 ± 0.3	1.7 ± 0.3
Ridge/Slope #2 (Head-slope of L.V.)	Forest	3.1 ± 0.3 (7.8 ± 0.7)	8.0 ± 3.2	17.5 ± 3.4	1.2 ± 0.3	1.3 ± 0.3
Ridge/Slope #3 (Ridge and slope of island peak)	Forest edge and open shrubland	2.3 ± 0.2 (5.4 ± 0.5)	28.1 ± 3.3	16.2 ± 5.2	1.1 ± 0.2	0.9 ± 0.2
Ridge/Slope #4 (Slope of L.V. northwest wall)	Forest	4.2 ± 0.2 (10.4 ± 0.9)	19.6 ± 6.0	16.1 ± 4.6	0.5 ± 0.2	0.9 ± 0.3
Cliff (northeast cliff and windward slope)	Windswept shrubland with herbs and grass	0.8 ± 0.1 (2.9 ± 0.2)	14.3 ± 4.8	75.0 ± 8.0	**0.7 ± 0.2**	**1.7 ± 0.2**

Table 2. Legacy brodifacoum residues from 2012 rat-eradication attempt, Desecheo Island, Puerto Rico. Animals in the table were live-harvested the week prior to the March 2016 bait application. Therefore, these residues persisted in the environment during the 4 years between bait applications, as indicated by their appearance in the biological food web in 2016. In addition to the animals containing residues from the 2012 bait application, there were several species that were sampled and did not contain legacy residues of brodifacoum (i.e., geckos, anoles, and hermit crabs; designated by "ND", or below detection levels, which were: 5.4 ppb for hermit crabs, 7.9 ppb for birds, 8.8 for arthropods other than hermit crabs; 11 ppb for rats and ameivas; 12 ppb for anoles and geckos).

Species	# of samples containing brodifacoum	Brodifacoum concentration (ppb)
Ameiva (*Ameiva exsul desechensis*)	3 of 20	27-34
Black rat (*Rattus rattus*)	2 of 23	56-58
Pearly-eyed thrasher (*Margarops fuscatus*)	1 of 1	134
Insect (18 cockroach individuals; Order Blattodea)	1 of 10	36
Gecko (*Sphaerodactylus levinsi*)	0 of 20	ND
Anole (*Anolis desechensis*)	0 of 20	ND
Hermit crab (*Coenobita clypeatus*)	0 of 3	ND

Table 3. Results of brodifacoum residues in animals harvested the week prior to the 2016 rat eradication attempt on Desecheo Island, Puerto Rico (thus **Pre-application** sampling shown here). The sample ID number corresponds to those listed in the full NWRC chemistry report (see Appendix); the brodifacoum concentration is reported in ng/g (nanograms/gram), which is equivalent to ppb (parts per billion); ND stands for not detected (i.e., brodifacoum levels were below the detection level of : 5.4 ppb for hermit crabs, 7.9 ppb for birds, 8.8 for arthropods other than hermit crabs; 11 ppb for rats and ameivas; 12 ppb for anoles and geckos), see additional footnotes. Note that most samples were analyzed multiple times, and we chose to use the highest values for each sample for interpretations.

Sample ID	Sample Collection Date	Species	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
Target Species							
S160511-85	3/15/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	56.0
						8/23/16	12.4*
S160511-86	3/15/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-87	3/15/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	58.1
						8/23/16	26.5*
						8/25/16	44.9
S160511-88	3/15/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-89	3/15/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
S160511-90	3/15/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-91	3/15/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-92	3/15/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-93	3/15/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND

Sample ID	Sample Collection Date	Species	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
						8/25/16	ND
S160511-94	3/15/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/23/16	ND
S160511-95	3/15/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/23/16	ND
S160511-96	3/16/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-97	3/16/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/23/16	ND
S160511-98	3/16/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-99	3/16/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-100	3/16/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-101	3/16/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-102	3/16/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-103	3/16/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-104	3/16/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-105	3/16/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-106	3/16/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND
						8/25/16	ND
S160511-107	3/16/16	Black rat (*Rattus rattus*)	Liver	1	PRE	8/19/16	ND

Sample ID	Sample Collection Date	Species	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
						8/25/16	ND
Non-Target Species							
S160511-110	3/15/16	Pearly-eyed thrasher (*Margarops fuscatus*)	Liver	1	PRE	9/13/16	134
						9/19/16	127
S160511-01	3/14/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
						8/29/16	ND
S160511-02	3/14/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
						8/29/16	ND
S160511-03	3/14/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
S160511-04	3/14/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
						8/29/16	ND
S160511-05	3/14/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
S160511-06	3/14/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
						8/29/16	ND
S160511-07	3/14/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	28.0*
						8/29/16	31.5*
						8/29/16	31.4*
S160511-08	3/14/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
						8/29/16	ND
S160511-09	3/15/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
						8/29/16	ND
S160511-10	3/15/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	27.0*
						8/29/16	26.4*
						8/29/16	27.4*
S160511-11	3/15/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
S160511-12	3/15/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
						8/29/16	ND

Sample ID	Sample Collection Date	Species	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
S160511-13	3/15/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/4/16	ND
S160511-14	3/16/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
						8/29/16	ND
S160511-15	3/16/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
						8/29/16	ND
S160511-16	3/16/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
						8/29/16	ND
S160511-17	3/16/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	34.0*
						8/29/16	41.8
						8/29/16	39.6
S160511-18	3/15/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/4/16	ND
S160511-19	3/16/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
						8/29/16	ND
S160511-20	3/16/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Liver	1	PRE	8/9/16	ND
						8/29/16	ND
S160511-109	3/17/16	Green Iguana (*Iguana iguana*)	Liver	1	PRE	8/9/16	ND
						8/29/16	ND
S160511-45	3/15/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-46	3/15/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-47	3/16/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-48	3/16/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-49	3/16/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-50	3/16/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-51	3/16/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND

Sample ID	Sample Collection Date	Species	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
S160511-52	3/16/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-53	3/15/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-54	3/16/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-55	3/17/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-56	3/17/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-57	3/17/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-58	3/17/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-59	3/16/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-60	3/17/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-61	3/17/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-62	3/16/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-63	3/17/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-64	3/14/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Whole body	1	PRE	8/3/16	ND
S160511-65	3/14/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-66	3/14/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-67	3/14/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-68	3/15/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-69	3/15/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-70	3/14/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND

Sample ID	Sample Collection Date	Species	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
S160511-71	3/15/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-72	3/15/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-73	3/14/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-74	3/16/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-75	3/15/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-76	3/15/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-77	3/15/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-78	3/15/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-79	3/15/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-80	3/15/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-81	3/16/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-82	3/16/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-83	3/15/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-84	3/15/16	Desecheo anole (*Anolis desechensis*)	Whole body	1	PRE	9/14/16	ND
S160511-32	3/16/16	Grasshoppers (Suborder Caelifera)	Whole body	3	PRE	8/11/16 9/14/16	ND ND
S160511-33	3/16/16	Grasshoppers (Suborder Caelifera)	Whole body	6	PRE	8/11/16 9/14/16	ND ND
S160511-34	3/16/16	Grasshoppers (Suborder Caelifera)	Whole body	3	PRE	8/11/16 9/14/16	ND ND
S160511-35	3/16/16			3	PRE	8/11/16	ND

Sample ID	Sample Collection Date	Species	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
		Grasshoppers (Suborder Caelifera)	Whole body			9/14/16	ND
S160511-36	3/16/16	Grasshoppers (Suborder Caelifera)	Whole body	3	PRE	8/11/16	ND
						9/14/16	ND
S160511-37	3/16/16	Grasshoppers (Suborder Caelifera)	Whole body	3	PRE	8/11/16	ND
						9/14/16	ND
S160511-38	3/17/16	Cockroaches (Order Blattodea)	Whole body	18	PRE	8/11/16	30.9
						9/14/16	36.1
						9/14/16	45.5
S160511-39	3/17/16	Spiders (Class Arachnida)	Whole body	8	PRE	8/11/16	ND
						9/14/16	ND
S160511-40	3/18/16	Spiders (Class Arachnida)	Whole body	9	PRE	8/11/16	ND
						9/14/16	ND
S160511-44	3/15/16	Scorpion (Class Arachnida)	Whole body	1	PRE	8/11/16	ND
S160511-41	3/17/16	Tropical hermit crab (*Coenobita clypeatus*)	Whole body	1	PRE	8/11/16	ND
						9/14/16	ND
S160511-42	3/17/16	Tropical hermit crab (*Coenobita clypeatus*)	Whole body	1	PRE	8/11/16	ND
						9/14/16	ND
S160511-43	3/17/16	Tropical hermit crab (*Coenobita clypeatus*)	Whole body	1	PRE	8/11/16	ND
						9/14/16	ND

ND = Not detected. For rat and ameiva liver samples that had an initial result of "ND." a second preparation was extracted to confirm the initial result (if sufficient tissue was remaining). Avian, gecko, and anolis samples with an initial result of "ND" were not retested due to the small amount of tissue available.

* = Observed brodifacoum concentrations were greater than the Detection Limit, but less than the Quantitation Limit. Care should be taken when evaluating results below the Quantitation Limit as the variability will be significantly greater than the variability observed in quality control samples.

Table 4. Carcasses recovered on Desecheo Island, Puerto Rico, following the 2016 rat eradication attempt. All rats and pearly-eyed thrasher carcasses found on or after April 7 were too far decayed to collect for further chemical analysis. In total, the number of carcasses found were 22 rats, 10 pearly eyed thrashers, 1 kingfisher, 1 kingbird, 1 ameiva, 1 eel, 1 hermit crab, and 1 macaque.

Species	Location of found carcass	Date carcass was found
Black rat (*Rattus rattus*)	N 18.38310, W 62.48594	3/22/2016
Black rat (*Rattus rattus*)	N 18.38310, W 62.48594	3/22/2016
Black rat (*Rattus rattus*)	N 18.38310, W 62.48594	3/22/2016
Black rat (*Rattus rattus*)	GPS 004	3/22/2016
Black rat (*Rattus rattus*)	West Valley base camp	3/23/2016
Black rat (*Rattus rattus*)	West Valley beach	3/23/2016
Black rat (*Rattus rattus*)	Beach near heli-pad	3/23/2016
Black rat (*Rattus rattus*)	West Coast by heli-pad	3/23/2016
Black rat (*Rattus rattus*)	West Coast by heli-pad	3/23/2016
Black rat (*Rattus rattus*)	West Coast by heli-pad	3/23/2016
Black rat (*Rattus rattus*)	West Valley beach	3/23/2016
Black rat (*Rattus rattus*)	Deflector #1 beach	3/23/2016
Black rat (*Rattus rattus*)	Deflector #2 beach	3/23/2016
Belted kingfisher (*Megaceryle alcyon*)	West coast, beach	3/23/2016
Eel	Near West Valley, beach	3/22-3/25 2016
Black rat (*Rattus rattus*)	West Valley	3/24/2016
Black rat (*Rattus rattus*)	West Valley	3/24/2016

Black rat (*Rattus rattus*)	Cliff #1	3/24/2016
Black rat (*Rattus rattus*)	Southeast coast, N 18.37876, W 67.47724	3/25/2016
Black rat (*Rattus rattus*)	West coast beach	3/25/2016
Black rat (*Rattus rattus*)	Long Valley, Ridge #2	3/25/2016
Pearly-eyed thrasher (*Margarops fuscatus*)	Long Valley, 50 m from shore	3/25/2016
Pearly-eyed thrasher (*Margarops fuscatus*)	N 18.38339, W 62.48587	3/30/2016
Pearly-eyed thrasher (*Margarops fuscatus*)	N 18.38267, W 62.48249	3/31/2016
Pearly-eyed thrasher (*Margarops fuscatus*)	N 18.38157, W 62.48301	3/31/2016
Black rat (*Rattus rattus*)	Heli-pad	4/7/2016
Black rat (*Rattus rattus*)	Deflector #2 (7 m from water's edge)	4/7/2016
Pearly-eyed thrasher (*Margarops fuscatus*)	Ridge 10	4/8/2016
Pearly-eyed thrasher (*Margarops fuscatus*)	Trail between West Valley and Long Valley	4/8/2016
Pearly-eyed thrasher (*Margarops fuscatus*)	Long Valley	4/9/2016
Rhesus macaque (*Macaca mulatta*)	Shoreline on north side of heli-pad	4/9/2016
Pearly-eyed thrasher (*Margarops fuscatus*)	East Valley	4/10/2016
Pearly-eyed thrasher (*Margarops fuscatus*)	East Valley	4/10/2016
Ameiva (*Ameiva exsul desechensis*)	Long Valley	4/26/2016

Pearly-eyed thrasher (*Margarops fuscatus*)	Long Valley	4/26/2016
Gray Kingbird (*Tyrannus dominicensis*)	Long Valley	4/26/2016
Hermit crab (*Coenobita clypeatus*)	Long Valley	4/26/2016

Table 5. Results of brodifacoum residues in animals live-harvested and found dead after brodifacoum-laced bait was first applied (March 18) during the 2016 rat eradication attempt on Desecheo Island, Puerto Rico (thus **Post-application** sampling shown here). The sample ID number corresponds to those listed in the full NWRC chemistry report (see Appendix); the brodifacoum concentration is reported in ng/g (nanograms/gram), which is equivalent to ppb (parts per billion); ND stands for not detected (i.e., brodifacoum levels were below the detection level of : 5.4 ppb for hermit crabs, 7.9 ppb for birds, 8.8 for arthropods other than hermit crabs; 11 ppb for rats and ameivas; 12 ppb for anoles and geckos), see additional footnotes. Note that most samples were analyzed multiple times, and we chose to use the highest values for each sample for interpretations.

Sample ID	Sample Collection Date	Species	Sampled Alive or Found Carcass	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
Target Species								
S160511-201	3/23/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	11500
							8/23/16	12700
S160511-202	3/23/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/19/16	8090
							8/23/16	7300
							8/23/16	6360
S160511-203	3/24/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	8480
							8/23/16	5300
S160511-204	3/23/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	14900
							8/23/16	11800
S160511-205	3/25/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	22600
							8/23/16	19900
S160511-206	3/24/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	7810
							8/23/16	9170
S160511-207	3/25/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	7450
							8/23/16	7500
S160511-208	3/22/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	18300
							8/23/16	20200

Sample ID	Sample Collection Date	Species	Sampled Alive or Found Carcass	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
S160511-209	3/25/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	14600
							8/23/16	10600
S160511-210	3/24/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	8790
							8/23/16	8930
S160511-211	3/23/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	9690
							8/23/16	7700
S160511-212	3/22/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	18500
							8/23/16	16200
S160511-213	3/23/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	9160
							8/23/16	8290
S160511-214	3/23/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	12000
							8/23/16	13500
S160511-215	3/22/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	26800
							8/23/16	27700
S160511-216	3/23/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	21100
							8/23/16	16900
S160511-217	3/22/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	15100
							8/23/16	14100
S160511-218	3/23/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	12400
							8/23/16	12600
S160511-219	3/23/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	13200
							8/23/16	18000
S160511-220	4/8/16	Black rat (*Rattus rattus*)	Carcass	Liver	1	POST	8/23/16	14100
							8/23/16	13000
Non-Target Species								
S160511-191	4/8/16	Pearly-eyed thrasher (*Margarops fuscatus*)	Carcass	Liver	1	POST	9/13/16	134
							9/15/16	127
S160511-192	4/8/16		Carcass	Liver	1	POST	9/13/16	1090

Sample ID	Sample Collection Date	Species	Sampled Alive or Found Carcass	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
		Pearly-eyed thrasher (*Margarops fuscatus*)					9/15/16	1520
S160511-193	4/10/16	Pearly-eyed thrasher (*Margarops fuscatus*)	Carcass	Liver	1	POST	9/13/16	1890
							9/15/16	1180
S160511-194	3/23/16	Belted kingfisher (*Megaceryle alcyon*)	Carcass	Liver	1	POST	9/13/16	2780
							9/15/16	1720
S160511-195	3/25/16	Pearly-eyed thrasher (*Margarops fuscatus*)	Carcass	Liver	1	POST	9/13/16	1340
							9/15/16	1330
S160511-196	3/26/16	Pearly-eyed thrasher (*Margarops fuscatus*)	Carcass	Liver	1	POST	†	†
S160511-197	3/26/16	Gray kingbird (*Tyrannus dominicensis*)	Carcass	Liver	1	POST	9/13/16	ND
S160511-198	3/30/16	Pearly-eyed thrasher (*Margarops fuscatus*)	Carcass	Liver	1	POST	9/13/16	1450
							9/15/16	2230
S160511-199	3/31/16	Pearly-eyed thrasher (*Margarops fuscatus*)	Carcass	Liver	1	POST	9/13/16	1740
							9/15/16	1630
S160511-200	3/31/16	Pearly-eyed thrasher (*Margarops fuscatus*)	Carcass	Liver	1	POST	8/23/16	703
							8/23/16	896
S160511-111	4/8/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Sampled Alive	Liver	1	POST	‡	‡
S160511-112	4/8/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Sampled Alive	Liver	1	POST	8/9/16	372
							8/29/16	335
							8/29/16	598
S160511-113	4/9/16		Sampled Alive	Liver	1	POST	8/9/16	344
							8/29/16	371

Sample ID	Sample Collection Date	Species	Sampled Alive or Found Carcass	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
		Desecheo ground lizard (*Ameiva exsul desechensis*)					8/29/16	386
S160511-114	4/9/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Sampled Alive	Liver	1	POST	8/9/16	314
							8/29/16	283
							8/29/16	527
S160511-115	4/9/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Sampled Alive	Liver	1	POST	8/29/16	355
							8/29/16	1100
S160511-116	4/9/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Sampled Alive	Liver	1	POST	8/9/16	787
S160511-117	4/10/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Sampled Alive	Liver	1	POST	8/9/16	565
							8/29/16	601
							8/29/16	608
S160511-118	4/10/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Sampled Alive	Liver	1	POST	8/9/16	466
S160511-119	4/11/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Sampled Alive	Liver	1	POST	8/29/16	542
							8/29/16	344
S160511-120	4/12/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Sampled Alive	Liver	1	POST	8/9/16	195
							8/29/16	163
							8/29/16	219
S160511-121	4/12/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Sampled Alive	Liver	1	POST	8/29/16	646
							8/29/16	515
S160511-122	4/12/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Sampled Alive	Liver	1	POST	8/9/16	248
							8/29/16	366
							8/29/16	236
S160511-123	4/13/16	Desecheo ground lizard (*Ameiva exsul desechensis*)	Sampled Alive	Liver	1	POST	8/9/16	230
							8/29/16	223
							8/29/16	270
S160511-190	4/26/16		Carcass	Liver	1	POST	8/29/16	844

Sample ID	Sample Collection Date	Species	Sampled Alive or Found Carcass	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
		Desecheo ground lizard (*Ameiva exsul desechensis*)					8/29/16	769
S160511-144	4/7/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	13.5*
S160511-145	4/8/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	ND
S160511-146	4/8/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	ND
S160511-147	4/8/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	ND
S160511-148	4/8/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	12.4*
S160511-149	4/8/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	ND
S160511-150	4/8/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	ND
S160511-151	4/8/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	ND
S160511-152	4/8/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	47.8
							9/19/16	45.6
S160511-153	4/8/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	14.1*
							9/19/16	ND
S160511-154	4/10/16	Desecheo dwarf gecko	Sampled Alive	Whole body	1	POST	8/4/16	70.0

Sample ID	Sample Collection Date	Species	Sampled Alive or Found Carcass	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
		(*Sphaerodactylus levinsi*)						
S160511-155	4/13/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	41.9
							9/19/16	45.4
S160511-156	4/13/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	18.6*
S160511-157	4/13/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	48.2
S160511-158	4/13/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	29.7*
							9/19/16	10.6*
S160511-159	4/13/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	50.6
S160511-160	4/13/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	150
							9/19/16	133
S160511-161	4/14/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	22.4*
							9/19/16	33.4*
S160511-162	4/14/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	77.1
							9/19/16	46.1
S160511-163	4/14/16	Desecheo dwarf gecko (*Sphaerodactylus levinsi*)	Sampled Alive	Whole body	1	POST	8/4/16	98.1
							9/19/16	177
S160511-124	4/7/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	ND
S160511-125	4/7/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	61.5
							9/19/16	67.8

Sample ID	Sample Collection Date	Species	Sampled Alive or Found Carcass	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
S160511-126	4/7/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	11.6*
							9/19/16	15.2*
S160511-127	4/7/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	37.8*
							9/19/16	36.9*
S160511-128	4/7/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	13.6*
							9/19/16	18.3*
S160511-129	4/7/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	29.5*
							9/19/16	37.4*
S160511-130	4/7/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	ND
S160511-131	4/7/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	46.6
							9/19/16	46.8
S160511-132	4/8/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	ND
S160511-133	4/8/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	ND
S160511-134	4/8/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	ND
S160511-135	4/8/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	22.6*
							9/19/16	20.8*
S160511-136	4/13/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	ND
S160511-137	4/13/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	203
							9/19/16	213
S160511-138	4/13/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	23.3*
							9/19/16	22.0*
S160511-139	4/13/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	198
							9/19/16	233

Sample ID	Sample Collection Date	Species	Sampled Alive or Found Carcass	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
S160511-140	4/13/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	133
							9/19/16	155
S160511-141	4/13/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	52.2
							9/19/16	51.1
S160511-142	4/13/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	ND
S160511-143	4/13/16	Desecheo anole (*Anolis desechensis*)	Sampled Alive	Whole body	1	POST	9/14/16	13.3*
							9/19/16	13.8*
S160511-164	4/7/16	Grasshoppers (Suborder Caelifera)	Sampled Alive	Whole body	6	POST	8/11/16	ND
							9/14/16	10.3*
S160511-165	4/7/16	Katydid (Suborder Ensifera)	Sampled Alive	Whole body	2	POST	8/11/16	ND
							9/14/16	ND
S160511-166	4/7/16	Katydid (Suborder Ensifera)	Sampled Alive	Whole body	2	POST	8/11/16	ND
							9/14/16	ND
S160511-167	4/8/16	Katydid (Suborder Ensifera)	Sampled Alive	Whole body	2	POST	8/11/16	ND
							9/14/16	ND
S160511-168	4/8/16	Katydid (Suborder Ensifera)	Sampled Alive	Whole body	2	POST	8/11/16	ND
							9/14/16	ND
S160511-169	4/8/16	Hawk moth (Family Sphingidae)	Sampled Alive	Whole body	2	POST	8/11/16	ND
							9/14/16	ND
S160511-170	4/8/16	Grasshoppers (Suborder Caelifera)	Sampled Alive	Whole body	5	POST	8/11/16	ND
							9/14/16	ND
S160511-171	4/8/16	Grasshoppers (Suborder Caelifera)	Sampled Alive	Whole body	2	POST	8/11/16	ND
							9/14/16	ND
S160511-172	4/8/16	Cockroaches (Order Blattodea)	Sampled Alive	Whole body	9	POST	8/11/16	52.8
							9/14/16	62.2
S160511-173	4/8/16	Grasshoppers (Suborder Caelifera)	Sampled Alive	Whole body	5	POST	8/11/16	ND
							9/14/16	ND

Sample ID	Sample Collection Date	Species	Sampled Alive or Found Carcass	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
S160511-174	4/8/16	Spiders (Class Arachnida) and 1 scorpion	Sampled Alive	Whole body	2+1	POST	8/11/16	ND
							9/14/16	ND
S160511-175	4/8/16	Insecta (4 Himiptera bugs, and 1 longhorn beetle, Order Coleoptera)	Sampled Alive	Whole body	4+1	POST	8/11/16	ND
							9/14/16	ND
S160511-176	4/10/16	Grasshoppers (Suborder Caelifera)	Sampled Alive	Whole body	1	POST	8/11/16	ND
							9/14/16	ND
S160511-177	4/10/16	Grasshoppers (Suborder Caelifera)	Sampled Alive	Whole body	4	POST	8/11/16	ND
							9/14/16	ND
S160511-178	4/12/16	Grasshoppers (Suborder Caelifera)	Sampled Alive	Whole body	5	POST	8/11/16	ND
							9/14/16	ND
S160511-179	4/12/16	Grasshoppers (Suborder Caelifera)	Sampled Alive	Whole body	5	POST	8/11/16	883
							9/14/16	1120
							9/14/16	1010
S160511-180	4/13/16	Spiders (Class Arachnida)	Sampled Alive	Whole body	7	POST	8/11/16	14.8*
							9/14/16	19.9*
S160511-181	4/13/16	Grasshoppers (Suborder Caelifera)	Sampled Alive	Whole body	3	POST	8/11/16	ND
							9/14/16	ND
S160511-182	4/13/16	Cockroaches (Order Blattodea)	Sampled Alive	Whole body	7	POST	8/11/16	977
							9/14/16	1580
S160511-183	4/13/16	Caterpillars (*Eunica monima*; Order Lepidoptera)	Sampled Alive	Whole body	60	POST	8/11/16	ND
							9/14/16	ND
S160511-184	4/10/16	Grasshoppers (Suborder Caelifera)	Sampled Alive	Whole body	2	POST	8/11/16	ND
							9/14/16	ND
S160511-185	4/10/16	Katydid (Suborder Ensifera)	Sampled Alive	Whole body	4	POST	8/11/16	ND
							9/14/16	ND
S160511-186	4/8/16		Sampled Alive	Whole body	1	POST	8/11/16	13.1*
							9/14/16	13.9*

Sample ID	Sample Collection Date	Species	Sampled Alive or Found Carcass	Tissue Type Analyzed	Number of Individuals in Sample	Pre or Post 2016 Application	Residue Analysis Date	Brodifacoum Concentration (ng/g)
		Tropical hermit crab (*Coenobita clypeatus*)					9/14/16	15.7*
S160511-187	4/8/16	Tropical hermit crab (*Coenobita clypeatus*)	Sampled Alive	Whole body	1	POST	8/11/16	8.3*
							9/14/16	9.3*
							9/14/16	13.8*
S160511-188	4/8/16	Tropical hermit crab (*Coenobita clypeatus*)	Sampled Alive	Whole body	1	POST	8/11/16	16.1*
							9/14/16	23.7
							9/14/16	24.8
S160511-189	4/26/16	Tropical hermit crab (*Coenobita clypeatus*)	Carcass	Whole body	1	POST	8/11/16	23.9
							9/14/16	36.6
							9/14/16	35.7

ND = Not detected. For samples that had an initial result of “ND” a second preparation was extracted to confirm the initial result (if sufficient tissue was remaining).

† = Sample was too decomposed to conduct residue analyses.

‡ = Sample was lost in the homogenizer and could not be retrieved to complete residue analysis.

* = Observed brodifacoum concentrations were greater than the Detection Limit, but less than the Quantitation Limit. Care should be taken when evaluating results below the Quantitation Limit as the variability will be significantly greater than the variability observed in quality control samples.

Table 6. Post-application animal samples (dead and live-harvested) collected after the bait applications on Desecheo Island (March/April 2016) and Palmyra Atoll (June 2011) and tested for brodifacoum residues. Both operations applied bait aerially, by helicopter, using the same pilot and methodology. Desecheo had 2-3 times the label application rate using Brodifacoum 25D: Conservation (dry formulation of bait), and Palmyra had 6 times the label application rate using Brodifacoum 25W: Conservation (wet formulation of bait). Live animal were sampled approximately 23-26 days after the first application; dead animals were sampled opportunistically beginning after the first application. Animals are arranged in rows within the table, by animal type (e.g., rat, bird, lizard, crab, insect), to make comparisons between the two islands more convenient. Numbers in parentheses were the number of samples (usually individuals, except for insects) that had detectable levels of brodifacoum. Palmyra values are from Pitt et al. (2015).

Desecheo (2-3x label appl. rate)			**Palmyra (6x label appl. rate)**	
Dead animal sampled	Brodifacoum (ppb)		Dead animal sampled	Brodifacoum (ppb)
Rat (22)	7500-27700		Rat (21)	9570-29000
PE Thrasher (8)	896-2780		Curlew (4)	1110-10500
Kingfisher (1)	1340		Turnstone (2)	2140-3100
Kingbird (1)	0		Plover (2)	841-1690
-	-		Tattler (1)	680
Ameiva (1)	844		-	-
Hermit crab (1)	37		-	-
Live animal sampled	Brodifacoum (ppb)		Live animal sampled	Brodifacoum (ppb)
Ameiva (12)	219-1100		-	-
Anole (13/20)	10-233		-	-
Gecko (14/20)	12-177		Gecko (3/5)	0-42
Hermit crab (3)	14-25		Hermit crab (4/5)	0-177
Insect (5/22)	10-1580		Insect (5)	153-227

Figures

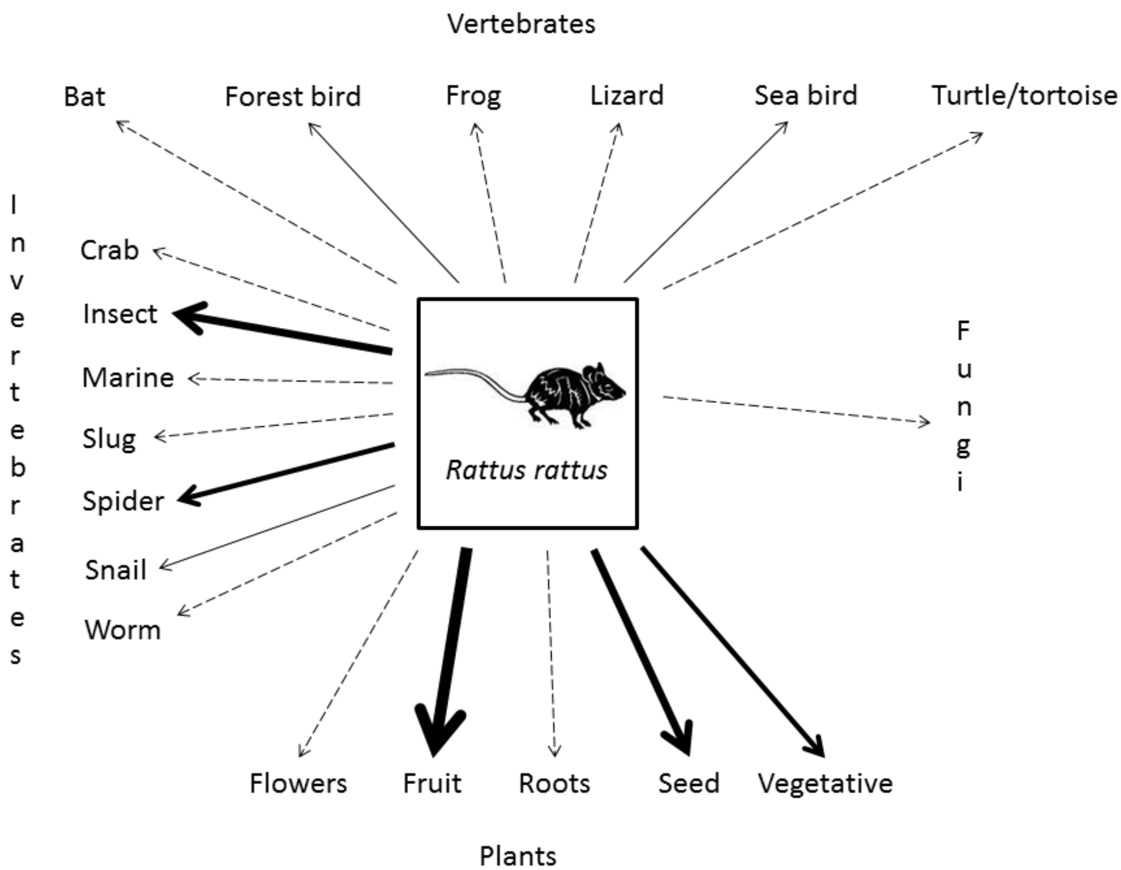

Figure 1. Organisms that *Rattus rattus* are known to consume on islands. The arrow's thickness indicates the average diet of *R. rattus* on Pacific islands, measured by the relative proportion of the food item in stomach contents and in some cases other indicators of diet; dashed lines indicate items that have been recorded but are least common in diets. In general, thickened solid arrows = common consumption of food item; thinnest solid arrows = generally infrequent by volume, but commonly consumed on some islands (i.e., snail, forest bird, sea bird); dashed arrows = uncommon consumption of food item (i.e., infrequent in most studies and islands). The vegetative category includes stems and leaves. All categories and relationships are based on reviewed literature (see Shiels et al. 2014). Figure taken from Shiels et al. (2014).

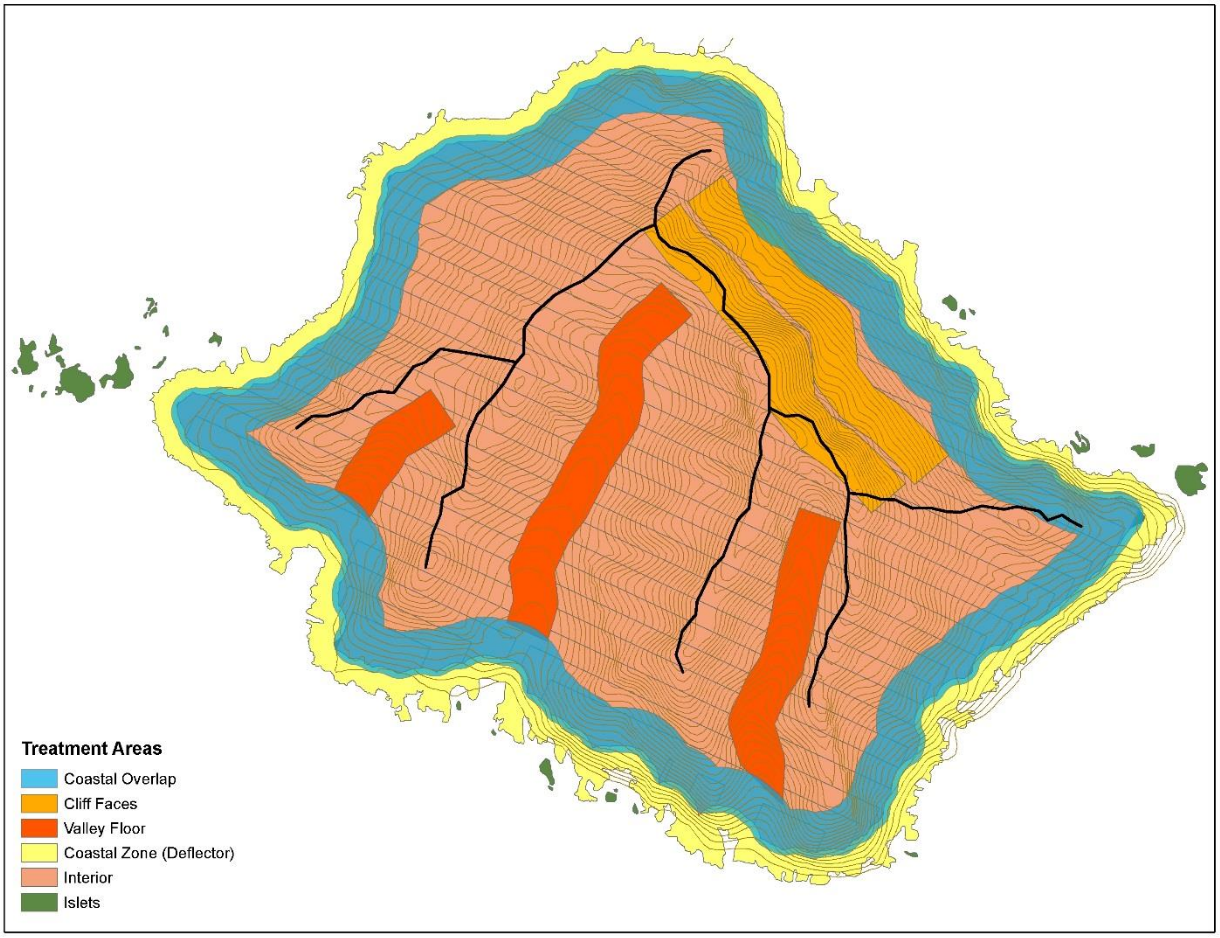

Figure 2. Map of Desecheo Island, Puerto Rico, outlining the different treatment zones for bait application. The entire island received two applications of Brodifacoum 25D: Conservation rodenticide bait in 2016 (March 18 and April 9). Bait application rates were 30 kg/ha for both applications for all parts of the island except the coastal overlap (blue), cliff faces (orange), and valley floors (red), which each received a total of 45 kg/ha during both applications. For orientation, there are three main valleys on the island shaded in red, where (left to right, or west to east) West Valley is the smallest and most western (also where camp was set up at the base), Long Valley is the middle valley, and East Valley is the eastern valley. The bulk of the monitoring was concentrated in the western half of the island. Map provided by Island Conservation.

	Pellet matrix	Change in shape	Presence of mold	Loss of volume
Condition 1 *Fresh pellets*	Identical to fresh bait	Identical to fresh bait	None	None
Condition 2 *Cracking*	<50% pellet matrix is or has been soft/moist	Distinct cylinder still; smooth sides may have been lost; cracking along surface of the pellet.	<50% bait pellets mold	Little or no volume lost
Condition 3 *Fracturing/flaking*	>50% bait matrix is or has been soft/moist	<50% pellet has lost distinct cylinder shape; fracturing or flaking of distinct pieces off the pellet.	>50% bait pellets have mold	Bait has lost some volume (<50%)
Condition 4 *Pile of dust*	100% of bait matrix is or has been soft	Pellets lost distinct cylinder shape and is no longer a pellet	>50% bait pellets have mold	Bait has lost some volume (>50%)
Condition 5 *Unorganized pile of dust*	100% of bait matrix is or has been soft	Pellet has completely lost distinct cylindrical shape and resembles a pile of dust particles	>50% bait pellets have mold	Bait has lost a significant amount of volume (>75%)

Figure 3. The Craddock (2004) bait degradation scale used to assess the condition of the bait each day that the baits were visited (e.g., daily for a week after each application) on Desecheo Island, Puerto Rico. Due to this index originally being developed for wet climates, we typically used the general descriptions: 1 = fresh pellets, 2 = cracking, 3 = fracturing/flaking, 4 = pile of dust, and >50% volume bait loss, 5 = unorganized pile of dust, and >75% volume bait loss.

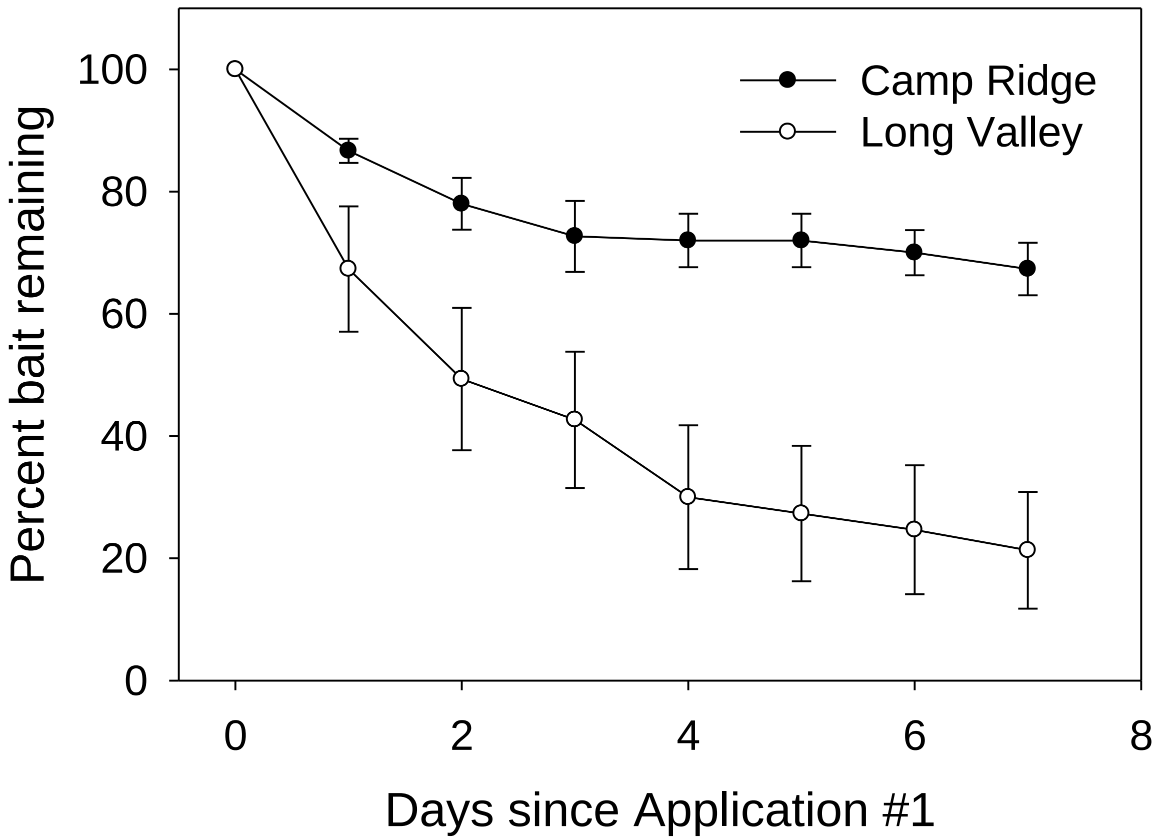

Figure 4. Mean ± SE percent bait remaining after Application #1 at two locations (n = 6 for each), Camp Ridge, which is grassland close to Ridge #3, and Long Valley, which is woodland adjacent to Long Valley #1 (valley bottom). These plots are 1 x 25 m, and each day the number of baits within them were counted. Note the drastic different between locations—the Camp Ridge plots retained >75% bait pellets the first week following application #1 whereas Long Valley plots retained just over 20% during the first week.

Figure 5. AS needs to add this figure (1m2 plots bait availability)..…

Figure 6. An adult ameiva (*Ameiva exsul desechensis*) triggers a motion-camera positioned to monitor brodifacoum bait pellets on Desecheo Island, March 2016. Notice the two green bait pellets at the base of pin-flag at the lower central position of the photo. Ameiva's rarely were photographed in contact with the bait, and were never documented consuming or removing the bait pellets.

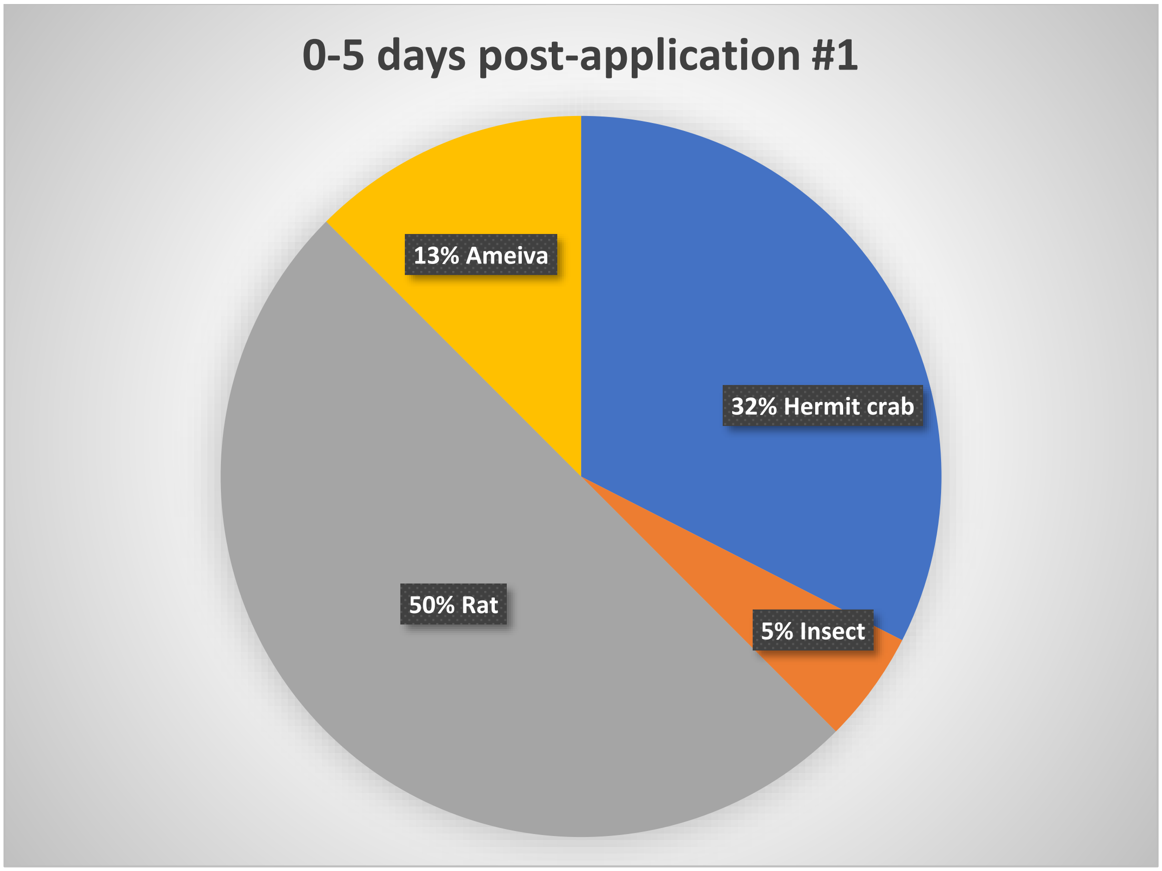

Figure 7. Percentage of all motion camera results when an animal was in contact with a bait pellet (e.g., touching, eating, removing) during the first 5 days (March 18-22) after bait application #1, on Desecheo Island, Puerto Rico. There was a total of 40 animals in contact with bait during this period (20 rats, 13 hermit crabs, 5 ameiva, and 2 insects), and these photos were captured on the following five sites (Cliff, Overlap 2, Deflector 1, Ridge 4, and Long Valley 1; see **Table 1** for site descriptions).

Figure 8. A black rat (*Rattus rattus*) triggers a motion-camera positioned to monitor brodifacoum bait pellets on Desecheo Island, March 2016. Notice the bait pellet the rat is nearly touching with its nose. Rat, being the target species, were commonly photographed consuming and removing the bait pellets for the first 5-7 days following the first bait application (March 18, 2016).

Figure 9. A black rat (*Rattus rattus*) triggers a motion-camera while eating a bait pellet on Desecheo Island, just hours after the first bait application on March 18, 2016.

Figure 10. A hermit crab (*Coenobita clypeatus*) triggers a motion-camera while approaching a bait pellet on Desecheo Island. Hermit crabs were the primary visitors and consumers of bait pellets after the first week following application #1.

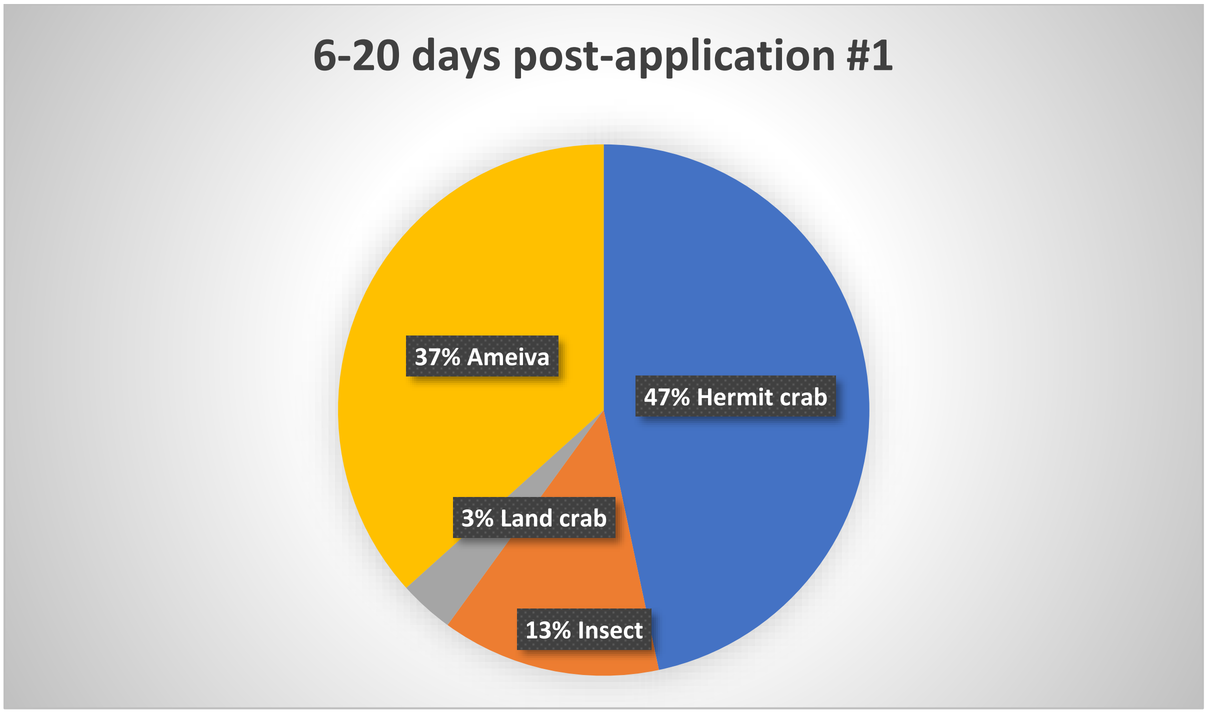

Figure 11. Percentage of all motion camera results when an animal was in contact with a bait pellet (e.g., touching, eating, removing) during days 6-20 (March 23-April 7) following bait application #1, on Desecheo Island, Puerto Rico. There was a total of 30 animals in contact with bait during this period (14 hermit crabs, 11 ameiva, 4 insects, and 1 black land crab), and these photos were captured on the following five sites (Cliff, Overlap 2, Deflector 1, Ridge 4, and Long Valley 1; see **Table 1** for site descriptions). Note that there were no rats photographed interacting with bait after 5 days, and rats were not photographed at all after 6 days following bait application #1.

Figure 12. A black land crab (*Gecarcinus ruricola*) triggers a motion-camera while consuming a bait pellet on Desecheo Island. Black land crabs were rarely observed, and only active at night, on Desecheo Island.

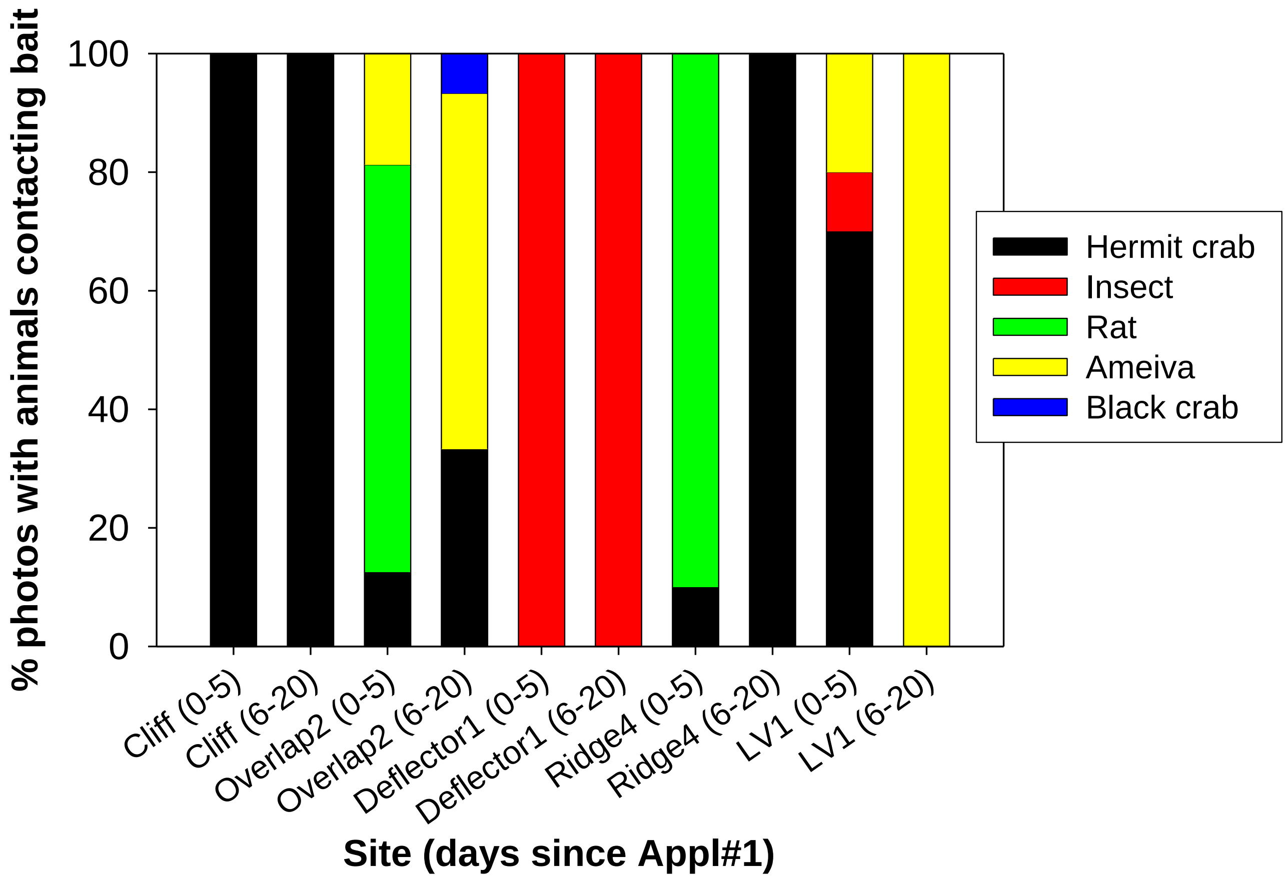

Figure 13. Percentage of all motion camera results, separated by site, depicting when an animal was in contact with a bait pellet (e.g., touching, eating, removing) during the initial 5 days (March 18-22), and days 6-20 (March 23-April 7), following bait application #1, on Desecheo Island, Puerto Rico. There was a total of 70 animals in contact with bait during this period (i.e., 40 during the initial 5 days, 30 from 6-20 days), and these photos were captured at the following five sites (Cliff, Overlap 2, Deflector 1, Ridge 4, and Long Valley 1; see **Table 1** for site descriptions). Note that there were no rats photographed interacting with bait after 5 days, and rats were not photographed at all after 6 days following bait application #1.

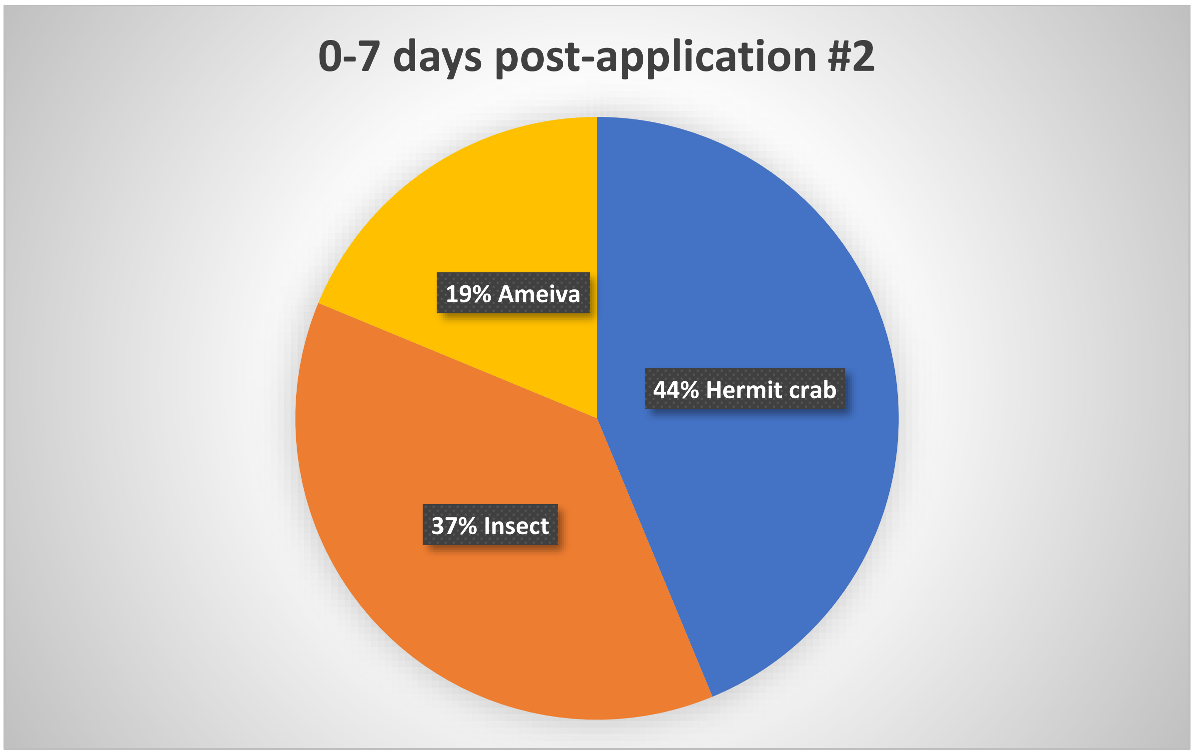

Figure 14. Percentage of all trail camera results when an animal was in contact with a bait pellet (e.g., touching, eating, removing) during days 0-7 (April 9-16) following bait application #2, on Desecheo Island, Puerto Rico. There was a total of 16 animals in contact with bait during this period (7 hermit crabs, 3 ameiva, 6 insects), and these photos were captured on the following five sites (Cliff, Overlap 1, Ridge 1, Ridge 4, and Long Valley 1; see **Table 1** for site descriptions).