**Appendix J: Protocols for Monitoring Operational Efficacy & Bait in the Environment**

**Overview**

Environmental monitoring will be conducted between March 09 and April 30 to support the rodent eradication project. An overview of the monitoring components, purpose, primary method to be used, and the lead for each component is shown in Table 1. An anticipated schedule of activities is shown in Table 2.



**Table 1.** Overview of monitoring activities to support the Desecheo rat eradication. Numbers refer to the sections following.

**Table 2.** Schedule of Activities(subject to change)



The core monitoring team will comprise: USDA-NWRC (1); U.S. Fish and Wildlife (3, 2 rotating); Island Conservation (4). Two law enforcement officers (FWS and DNER) will be continuously on Desecheo between March 08 and April 16, and will accompany staff on the shorter trips after April 16. Additional personnel will be available from the FWS and IC as needed for the monitoring trips: Mar 30-31; April 21-22; April 28-30.

1. **Rainfall Monitoring**

The bait product chosen for use during the operation is specially formulated for regions having a dry climate and is not designed to withstand heavy rainfall, but to break down quickly in the rain. As such, bait pellets are susceptible to degradation in the event of significant rainfall. The operational window was specifically chosen for the relatively dry climate experienced on Desecheo at that time of year, but it is not impossible that a rain event occur during the operation.

1. **MONITORING GOAL**

The goal of monitoring rainfall is to document any precipitation which might adversely affect the performance of the product and jeopardize the success of the eradication.

1. **METHODOLOGY**

On January 24 2016, a digital rain gauge was installed above the helipad, in the open grassland area.

1. Rainfall data will be downloaded on each trip to Desecheo prior to application, and at least once per week until personnel leave the island (end April).
2. Rainfall data will be stored, organized, and reviewed every time it is downloaded to the computer to allow constant monitoring and detect abnormal conditions should they occur.
3. **ANALYSIS AND REPORTING**

Rainfall data will be summarized and reported to the Operations Section Chief after each download.

1. **Monitoring Bait in the Environment**

Two aerial broadcast application periods will be made during the eradication attempt. Each application period will consist of three (3) aerial broadcast applications to specific island features conducted on the same day (if weather conditions remain favorable) (see *Figure 1*).

1) An application targeting the coastal zone of the island will be applied at a rate of 30 kg/ha (66 lbs/acre), using a deflector to direct bait away from the water’s edge.

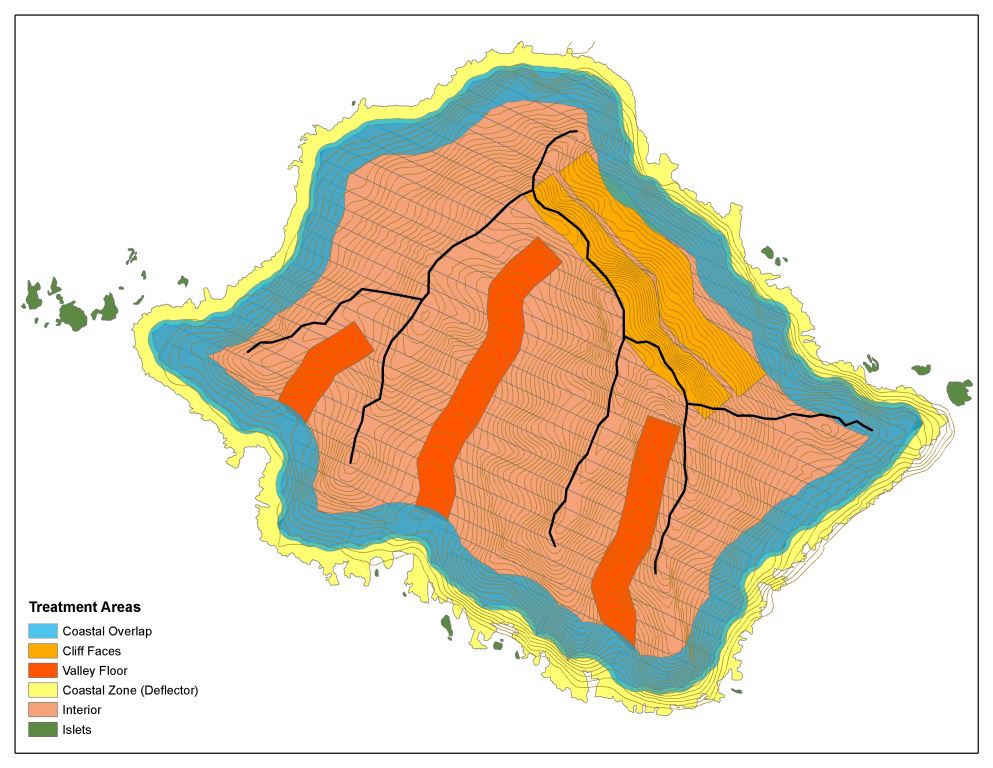
2) An application targeting the interior island and offshore islets will be applied at a rate of 30 kg/ha (66 lbs/acre).

3) An additional application of bait targeting the coastal/interior zone overlap, valley floors and interior cliff faces will be applied at a rate of 15 kg/ha (33 lbs/acre).

At points where flight lines overlap, the amount of bait applied might locally exceed the prescribed application rate. This could occur along adjacent borders of parallel swaths, at the end of swaths where they intercept the swaths created by shoreline baiting, or adjacent to areas missed during the initial baiting operations and subsequently rebaited, as indicated by the GPS flight path data.

The **planar area** (2-dimensional) of Desecheo Island is **116.1 ha** (not including offshore islets).The 3-dimensional surface area of Desecheo Island is **132.9 ha** (not including offshore islets), approximately 13% larger than the planar area. The application rates above specify the amount of bait delivered on the ground or to the 3-dimensional surface area of the island. Therefore, the bucket calibration (sowing) rate should be approximately 34 kg/ha and 17 kg/ha.

The interval between broadcast application periods will be approximately 24 days, but depending on local weather conditions or logistical considerations, could occur anytime between 17 and 66 days.



**Figure 1.** Proposed estimated bait application treatment zones for rodent eradication, Desecheo Island, 2016. Black lines are ridgelines which can also be used as trails.

* 1. **Bait Application Rate (density on the ground)**

1. **MONITORING GOAL**

To estimate the bait application rate (density on the ground) in kg/ha at different sites representative of different habitats and/or treatment zones for the 1st and 2nd aerial bait broadcast.

1. **METHODOLOGY**

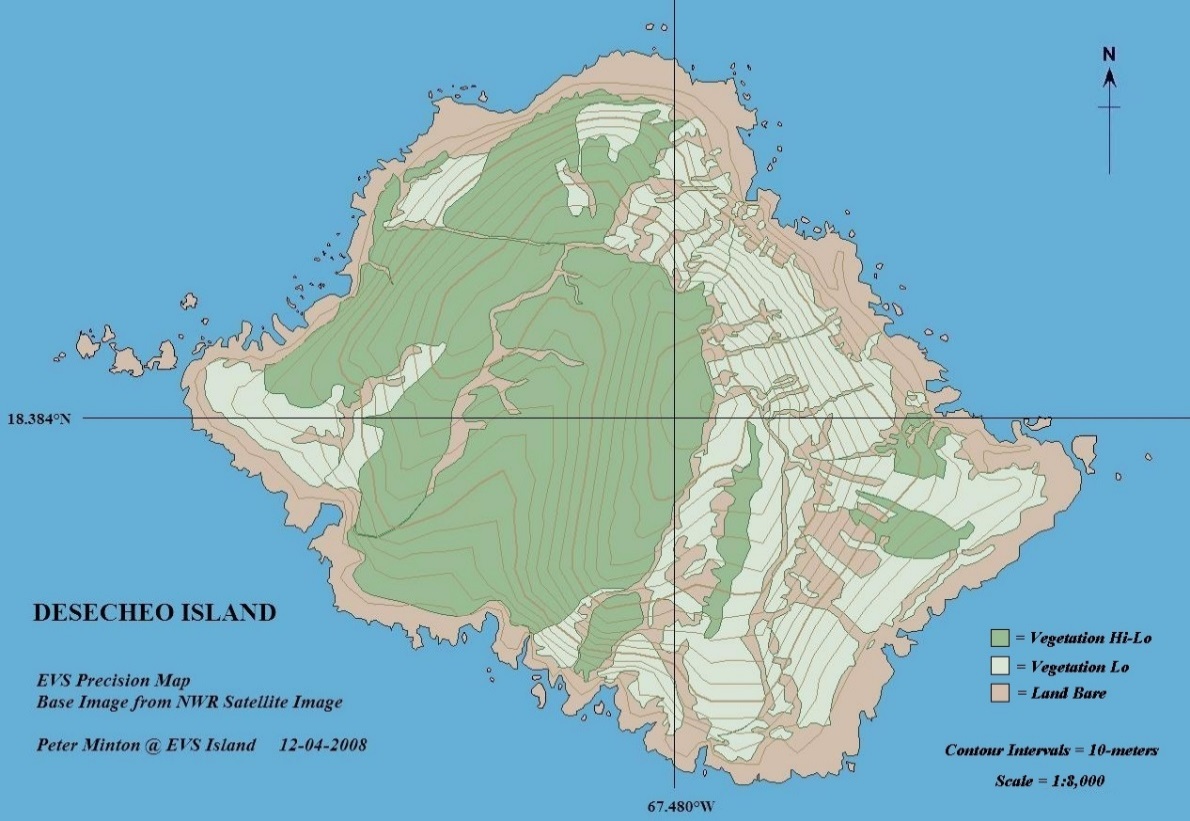
A circular hoop sampling method (1 m2) will be used to estimate bait density on-the-ground at different aerial broadcast treatment zones. At an average broadcast rate of 30 kg/ha, with an average mass of about 2.12g - 2.3g for each bait pellet, each 1m2 circular plot would be expected to have about 1.3 – 1.4 pellets. Areas with higher application rates according to the bait label can been expected. For example, for areas of 45 kg/ha the number of pellets should be about 1.96 pellets/m2 (if pellet mass = 2.3g) and 2.1 pellets per m2 (if pellet mass = 2.12g).

1. Prior to the 1st aerial bait broadcast, five different representative habitat types and/or treatment zones will be identified (**sample sites**), and marked with flagging tape and a GPS location (*Figure 1*, *Appendix 1*). The locations of the monitoring plots will be placed at random around existing trails/transects.
2. On each day of aerial bait broadcast, each sample site will be visited as soon after aerial bait broadcast as possible. Priority will be given to sampling in the valley floors where crab competition for bait will be highest; these plots should be sampled within 30 minutes of bait application on the ground to reduce inaccuracies caused by crabs. At the beginning of the day, personnel will be located close to valley plots in order to respond quickly.
3. Multiple samples will be collected from each sample site (Table 3) using a circular PVC hoop each 1 m2 (**sample plot**). At each sample site, the hoop will be randomly dropped from shoulder height, and marked with a pin flag in the middle.
4. The bait pellets inside the 1 m2 area will be counted and the number recorded.
5. The mass of each bait pellet is approximately 2.1 - 2.3 g; an estimate of pellet mass will be obtained from the bait product after arrival in Puerto Rico.

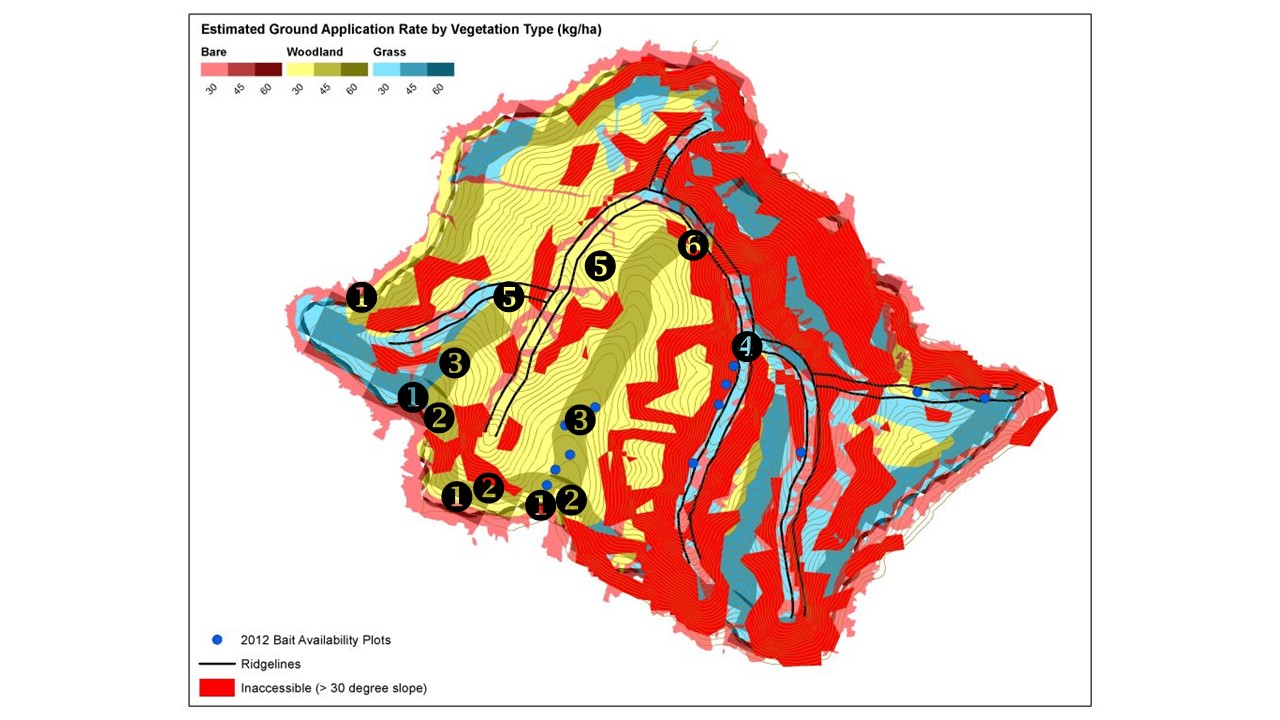
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Habitat** | **Bait treatment type** | **Location no. (Fig. 2)** | **No. sample sites** | **No. sample plots per site** | **Total no. sample plots** |
| Shrubland | Coastal zone with deflector | 1 | 3 | 15 | 45 |
| Shrubland | Coastal zone overlap | 2 | 3 | 15 | 45 |
| Woodland | Valley floor | 3 | 2 | 15 | 30 |
| Shrubland | Interior (ridgelines) | 4 | 2 | 15 | 30 |
| Woodland | Interior, valley slopes | 5 | 2 | 15 | 30 |
| Cliff faces | Interior | 6 | 1 | 15 | 15 |
| **TOTAL** | | **6** | **13** | **90** | **195** |

**Table 3.** Number and approximate location of sample plots to be monitored across representative habitats on Desecheo (see also Fig. 2).

**Figure 2.** Desecheo Island monitoring sites. **(A)** Topographical map showing primary vegetation zones. **(B)** Approximate locations of possible sample sites (1-6) representing different habitats and treatment zones described in *Table 1*, and showing ridges, inaccessible areas, and the bait monitoring plots used in 2012. **(C)** Representative photographs of the potential sample site habitats.



**(A)**



**(B)**

**(C)** Representative photographs of the potential sample site habitats, refer to (B) for locations.



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**ANALYSIS AND REPORTING**

Bait density on-the-ground will be summarized using descriptive statistics (e.g. mean, SD, N, range, etc). While sample sizes will be small, attempts will be made to describe the results as: (1) mean (SD) across habitat types and treatment zones for the whole island (not including offshore islets); (2) per habitat type; (3) per treatment zone. Bait density data will be made available to USFWS and Island Conservation at the end of each day for which these data were collected; this will enable time-sensitive adjustments to be made by the operational crew, and further maximize efficiency and project success and goals.

* 1. **Bait Availability**

In order to track the availability of bait to rats over time following the aerial broadcast of bait, three monitoring methods will be implemented:

1. Bait availability over time (daily presence/absence pellet monitoring)
2. Bait fate (monitoring individual pellets with remote cameras)
3. Bait degradation (pellet deterioration over time)
   * 1. **Bait Availability Over Time**
4. **MONITORING GOAL**

To provide an indicator of efficacy of the operation, and inform baiting decisions. For example, after the 1st bait application if bait pellets disappear more rapidly than expected, the timing of the second aerial broadcast could be modified. Two methods will be used to inform the aerial baiting operation:

1. Daily count of bait pellets in 1m2 plots across habitats
2. Daily count of bait pellets in 25m2 plots in woodland and cactus shrubland
3. **(1) METHODOLOGY - Daily pellet count in 1 m2 plots:**

The bait density sample plots will be used for subsequent bait availability monitoring.

1. Following counts of pellets in each bait density sample plot (see Section 2.1) two (2) bait pellets will be placed within 5 cm of the pin flag in each sample plot.
2. Each sample plot will be visited on each subsequent day for a period of up to seven (7) days or earlier if bait disappears.
3. At each visit, the number of bait pellets remaining at the pin flag in each sample plot will be recorded, and the bait condition will be recorded according to the Craddock Scale (see Section 2.2.3).

If time is limited, sampling plots may be prioritized as follows: 1) valley floor, 2) interior valley slopes and ridgelines, 3) coastal zone.

**(2) METHODOLOGY - Daily pellet count in 25 m2 plots:**

1. Two days prior to baiting operations, 12 bait availability transects will be established: 6 transects in the Long Valley (representing woodland) and 6 transects along the central ridge (representing cactus shrubland) (Fig. 2(B) (4)).
2. Transects will measure 25 m x 1 m; the perimeter will be delineated using pin flags spaced every 5 meters.
3. On the day of aerial broadcast, 2 personnel will be deployed to each site. Once the area has been aerially baited, personnel will search each transect and markeach pellet with a pin flag.
4. The target bait density on-the-ground will be 30 kg/ha, which is equivalent to 33 pellets (with a mass of 2.3 g per pellet).
5. Any pellets occurring within each transect above the 33 pellets will be removed and dispersed outside each transect; associated flags will also be removed.
6. If too few pellets are found within each transect, pellets should be added to each transect (collected from the adjacent area) to make 33 pellets and each marked with a flag.
7. Each transect will be monitored daily at approximately the same time until all pellets have disappeared.
8. Any flags found without pellets below them will be removed.
9. The number of pellets remaining in each transect will be recorded on data sheets.
10. **ANALYSIS AND REPORTING**

Bait availability will be summarized using descriptive statistics (e.g. mean, SD, N, range, etc). While sample sizes will be small, attempts will be made to describe the results as: (1) mean (SD) across habitat types and treatment zones for the whole island (not including offshore islets); (2) per habitat type; (3) per treatment zone. A bait availability curve over time will be produced every five (5) days from each aerial broadcast operation, and in a final report. Bait availability data will be made available to USFWS and Island Conservation at the end of each day for which these data were collected; this will enable time-sensitive adjustments to be made by the operational crew, and further maximize efficiency and project success and goals.

* + 1. **Bait Fate (bait visitation and consumption)**

1. **MONITORING GOAL**

To evaluate efficacy of the operation and potential impacts to the environment by monitoring the frequency of bait visitation and consumption by rats and by non-target species.

1. **METHODOLOGY**
2. Prior to the 1st aerial bait broadcast, a single motion-sensing camera will be placed within each of the bait monitoring sample sites and distributed among the key habitats (e.g. six (6) in interior sites and six (6) in coastal sites).
3. Cameras will be placed about 0.5 m off the ground, and fixed to trees or rocks (in shrubland) and marked with flagging tape and a GPS location.
4. Each camera will be programmed to detect motion across 24 hours (day and night).
5. On the day of aerial bait broadcast, two (2) bait pellets will be placed about 1 to 1.5 m in front of each camera.
6. Each subsequent day, the bait will be monitored.
7. After seven (7) days or until the bait pellet disappears, the SD card will be removed and data downloaded.
8. The camera will be moved to a different bait pellet if the bait pellet disappears before seven (7) days.

**ANALYSIS AND REPORTING**

Images will be downloaded from the SD camera cards about every seven (7) days. Initial screening of some cards may be carried out by field staff on Desecheo. USDA personnel will screen and analyze the photos at the USDA-NWRC, Colorado at the end of each baiting operation. Data collected will include: the types of animals visiting the baits, date and time bait removal occurred, etc.

* + 1. **Bait Degradation (Bait Longevity)**

1. **MONITORING GOAL**

To provide an indicator of efficacy of the operation by evaluating the effect of ambient environmental conditions on the persistence of bait pellets over time (without predation by rats, crabs, and other large vertebrates).

The break-down of bait as a result of climatic conditions and microbial and invertebrate activity (excluding crabs) will be documented through the use of bait degradation cages which exclude rats, crabs, and other large vertebrates. Adverse weather (as documented by rainfall monitoring) coupled with a rapid breakdown in bait pellets could potentially explain poor performance of the bait product and contribute to a failed operation. The condition of pellets will be gauged according to a bait degradation scale (Craddock Scale, Table 3) developed in New Zealand for the specific purpose of quantifying bait weathering.



**Figure 3.** Bait degradation monitoring exclusion cages, Desecheo, March 2012.

**METHODOLOGY**

Bait Degradation – exposed bait pellets

1. The bait pellets monitored for bait availability (see Section 2.2.1 (b)) will also be monitored for bait degradation.
2. Pellets will be monitored daily for seven days at approximately the same time of day.
3. Pellets will be visually inspected and each cage will be assigned a pellet score according to Craddock’s scale (Fig. 4) and recorded on the data sheet.
4. Each pellet will be scored individually for condition, and a photograph taken from a standard distance of 10 cm.

Bait Degradation – exclusion cages

1. Up to 16 bait degradation cages will be installed prior to the 1st aerial bait broadcast in the same areas as the bait availability sample sites and distributed within interior and coastal zones. Cages will be secured against rodents and crabs by stacking rocks along the outer edge of each cage. The location of each cage will be recorded with GPS.
2. On the day of the 1st aerial bait broadcast, two bait pellets are placed in the middle of each cage.
3. On the day of the 2nd aerial broadcast, an additional two pellets will be placed in the middle of each cage, separated and distinguishable from pellets placed after the 1st broadcast if they still remain.
4. Pellets will be monitored daily for seven days at approximately the same time of day.
5. Pellets will be visually inspected and each cage will be assigned a pellet score according to Craddock’s scale (Fig. 4) and recorded on the appropriate data sheet.
6. Each pellet will be scored individually for condition, and a photograph taken from a standard distance of 10 cm.
7. Photograph numbers will be recorded on data sheets.

1. **ANALYSIS AND REPORTING**

A bait degradation curve can be calculated for all baits, as well as for key habitats; (1) valley floor, (2) shrubland ridges, (3) coastal zones.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Condition | 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  *Soft pellets* | <50% pellet matrix is or has been soft/moist | Distinct cylinder still; smooth sides may have been lost | <50% bait pellets mold | Little or no volume lost |
| Condition 3  *Mush pellets* | >50% bait matrix is or has been soft/moist | <50% pellet has lost distinct cylinder shape | >50% bait pellets have mold | Bait has lost some volume (<50%) |
| Condition 4  *Pile of mush* | 100% of bait matrix is or has been soft | Pellets lost distinct cylinder shape & resembles a pile of mush with some grain particles in matrix showing distinct separation from main pile | >50% bait pellets have mold | Bait has lost some volume (<50%) |
| Condition 5  *Disintegrating pile of mush* | 100% of bait matrix is or has been soft | Pellet has completely lost distinct cylindrical shape and resembles a pile of mush with >50% of the grain particles in the bait matrix showing distinct separation from each other and the main pile | >50% bait pellets have mold | Bait has lost a significant amount of volume (>50%) |
| Condition 6  *Bait gone* | Bait is gone or is recognizable as only a few separated particles of grain or powder | Bait is gone or is recognizable as only a few separated particles of grain or powder | Bait is gone or is recognizable as only a few separated particles of grain or powder | Bait is gone or is recognizable as only a few separated particles of grain or powder |

**Figure 4.** Craddock Scale of bait degradation in the Pestoff product rodent bait. Source: *Craddock, P. (2004). Environmental breakdown and soil contamination by Pestoff poison bait (20ppm brodifacoum) at Tawharanui Regional Park, north of Auckland- Winter 2003 trial. Unpublished report for Northern Regional Parks, ARC.*

1. **Monitoring Brodifacoum in the Environment**

Exposure to brodifacoum from rodent bait can be primary (directly consuming bait), or secondary/tertiary (consuming contaminated prey). The aim of monitoring and sampling rats, lizards, birds, and invertebrates on Desecheo Island during baiting operations is to: (1) provide an indicator of operational efficacy (rat mortality); (2) inform potential unintentional impacts to native species (lizard, bird mortality); (3) indicate the potential transfer of brodifacoum within the island’s ecosystem.

These parameters will be investigated by:

Systematic and opportunistic searches for vertebrate carcasses (rodent and other vertebrates)

Collection of live rats, lizards, and invertebrates for brodifacoum residue analyses

Collection of carcasses for residue analyses

Motion cameras identifying animals taking/consuming bait (described above)

In addition, removal and disposal of rodent carcasses at least twice per week, until three (3) weeks have passed since the last broadcast application, is a requirement of the supplemental bait label and will help to decrease carcass availability to non-target consumers. Searches for vertebrate carcasses (rats, lizards, birds) will be conducted on the island before, during, and after each aerial bait broadcast.

* 1. **Carcass Searches**

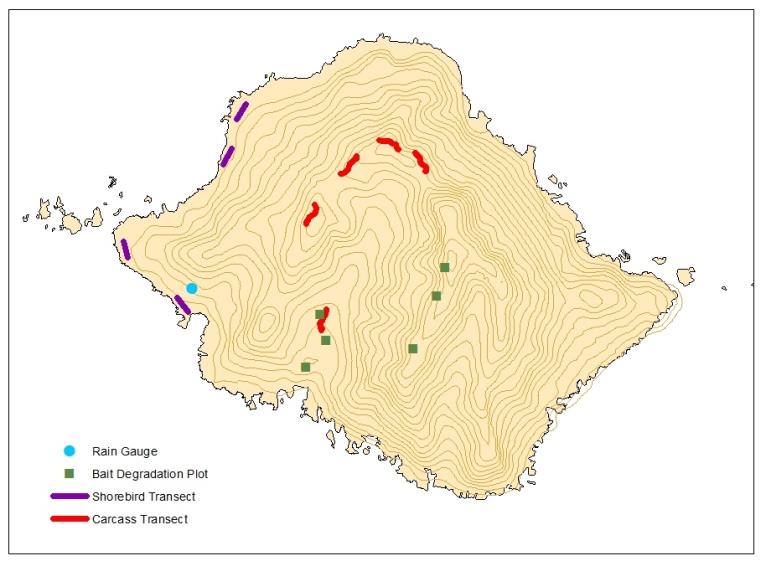
1. **METHODOLOGY**
2. **SYSTEMATIC SEARCHES**

Establish transects

1. Transects will be identified and marked prior to the 1st aerial bait broadcast.
2. Transects will be located on regularly used trails and representative of sample zones (interior/coastal) which may include: along the NW/SW beaches, in West and Long Valleys, on the trail up to the Top Camp.
3. Transects will measure a minimum of 50 meters, but will be longer where possible.
4. Transects will be established using GPS units and marked with flagging every ten meters.

Transect surveys

1. Transect searches for rodent and other vertebrate carcasses will occur simultaneously.
2. Carcass searches will occur before, during, and after each aerial bait broadcast.
3. *Before 1st aerial bait broadcast*: transects will be searched a minimum of two times up to two days before bait application.
4. *Before 2nd aerial bait broadcast*: transects will be searched at least once up to two days before bait application.
5. *After each aerial bait broadcast*: transects will be searched for carcasses starting four (4) days after each aerial bait broadcast and subsequently twice per week for three (3) weeks after the 2nd aerial bait broadcast.
6. Transects will be walked slowly while visually scanning the ground for any vertebrate carcasses approximately 2-2.5 meters on either side of the transect line (4-5 m swath).
7. Search effort (time start, time finish) will be recorded, and search route tracked with GPS.
8. Carcasses found will be recorded on the appropriate data sheet and collected (see sample collection and storage section below).



**Figure 5.** Desecheo Island showing the 2012 locations of the bait degradation cages and carcass transects. Carcass transects were on the main ridgeline trail.

1. **OPPORTUNISTIC SEARCHES**

Opportunistic carcass searches will take place any time that team members travel across the island. As it is highly likely that carcasses will be encountered outside of the designated monitoring sites, opportunistic carcass searches allow the monitoring team to document additional finds.

1. A GPS location and time will be recorded at the start and end of any hike across the island for a reasonable distance, to allow for search-effort calculations.
2. The carcass of any rat found outside of the transect searches will be recorded on the appropriate data sheet and collected (see sample collection and storage section below).
3. **ANALYSIS AND REPORTING**

The number of carcasses encountered can be quantified as a function of the area traveled and time taken to give a search effort. Results (number of carcasses, search effort) will be provided to USFWS and Island Conservation at the end of each day for which these data were collected; this will enable time-sensitive adjustments to be made by the operational crew, and further maximize efficiency and project success/goals.

* 1. **Brodifacoum Residue Analyses**

Monitoring unintentional environmental impacts on Desecheo will focus on the analysis of brodifacoum residue in focal parts of the food web by sampling pre- and post-aerial bait broadcast. Residue sampling will focus on rats, lizards, and insects to assess both primary and secondary (as potential prey items) impacts. The species are selected for residue analysis because of their likelihood to directly consume the brodifacoum bait, or consume brodifacoum indirectly by either scavenging a carcass (e.g. ameiva) or ingesting insects that had consumed the rodenticide bait (e.g. anole, gecko). Pre-application residue sampling will not only form baseline concentrations for which post-application monitoring should exceed if these animals are ingesting (directly or indirectly) brodifacoum, but will also help to establish if there are legacy residues residing in these animals from the 2012 brodifacoum bait applications. The slightly larger sample size for pre-application residue analysis for lizards is to improve chances of detecting legacy (from 2012) residues in each lizard population if such 2012 residues are indeed present.

* + 1. **Live Collection of Rats, Lizards, and Invertebrates**

1. **METHODOLOGY**

Live Capture Protocols

Live animals of each of the Desecheo anole, Desecheo ameiva, Desecheo gecko, and invertebrates will be collected before the 1st aerial bait broadcast to assess baseline residue levels.

Live animals of each species (as above) and invertebrates will subsequently be collected just prior to and in the seven days following the 2nd aerial bait broadcast.

1. Live rats will only be collected before the 1st aerial bait broadcast, but any moribund rats that can be captured by hand will also be collected.
2. Ameivas and anoles will be captured with a pole with a noose at the end, geckos will be captured by hand.
3. Reptiles will be captured early in the morning just after sunrise (0700-0900 hr) and late afternoon just before sunset (1600-1700 hr) when animals are more sluggish.
4. Rats will be collected with live Tomahawk traps because of the risk of capturing Desecheo ameiva and land crabs with snap-traps.
5. Insects will be captured by hand, with nets, with pan traps, or baited vials. Sunken pit-fall traps cannot be used because of the risk of below-ground UXOs. Each invertebrate sample must be at least 2 g in mass (total 20 g). Invertebrate collection will attempt to focus on potential lizard prey species (ants, termites, beetles, caterpillars, grasshoppers, any large dipteran), and each sample can comprise a mixture of different insects.
6. Where feasible, attempts will be made to collect samples across habitats and/or treatment zones.

Euthanasia Protocols

1. All live-captured animals will be euthanized on-site according to AVMA (2013) guidelines.
2. Rats will be held in live-traps, anesthetized using isoflurane (USDA/NWRC SOP: FP/HI 002.00), and cervical dislocation will be administered when the animal is unconscious and breathing is shallow. The trap containing the live rat will be placed into a 60 cm x 60 cm plastic ziplock bag with 1-2 cotton balls moistened with isoflurane. Because the large ziplock bags are clear plastic, the animals can be easily monitored. Once unconscious (usually within 30-90 seconds), the rat will be removed from the bag and trap, and euthanized by cervical dislocation
3. Lizards will be restrained by hand or noose and euthanized by manually applied blunt force trauma to the head (AVMA 2013). An alternative euthanasia technique that may be practiced on the lizards is to first immobilize them with isoflurane, and then either delivering the blow to the head or administer cervical dislocation.
4. Only USDA-Wildlife Services staff will conduct euthanasia using Isoflurane.
5. All outlined procedures involving animals, including euthanasia has been (currently in final review) approved (as QA-2588) by USDA/NWRC IACUC and Quality Assurance Committees.

Carcass Collection

1. Up to 30 rat carcasses, collected post-bait application, will be collected for brodifacoum residue analysis.
2. All non-target carcasses (reptiles, birds) found at any time will be collected and stored for residue analysis at USDA-NWRC.

Sample Collection and Storage

1. Whole animal samples (euthanized animals, carcasses, invertebrates) will be placed by gloved hand in a ziplock bag and the bag labelled with date, collection site, and species. All data will be
2. Samples will be kept cool on Desecheo (in coolers with ice) until shipment off-island.
3. In Puerto Rico, samples will be freeze-stored at USDA’s Cabo Rojo facilities (contact: Fred Soltero). USDA biologist Jose Rojas could collect samples from the appropriate boat dock and place in the freezer during regular working hours during the week, until the samples exported to the USDA-NWRC laboratory for assay.
4. Shipment of samples to the NWRC laboratory will be managed by USDA-Wildlife Services personnel.

Disposition of Carcasses After Sampling

1. Additional rat carcasses that are not required for laboratory analysis will be marked in the field (e.g. right ear snipped off, sharpie) to avoid double counting, and buried under rocks to reduce the risk of non-target scavengers.
2. If whole animals of native species remain after laboratory analyses, efforts will be made to deposit them at an institution where they can be of further public use (e.g. museum, university collection).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample/Species** | **Sample type** | **Sample period** | | **Total no. samples** |
| **Pre-bait exposure** | **Post-bait exposure** |
| **Mar 09-14** | **April 4-16** |
| Desecheo anole  (*Anolis desechensis*) | Live whole animal | 20 | 15 | 35 |
| Carcass | as available | as available |  |
| Desecheo Ameiva  (*Ameiva desechensis*) | Live whole animal | 20 | 15 | 35 |
| Carcass | as available | as available |  |
| Desecheo gecko  (*Sphaerodactylus levinsi*) | Live whole animal | 20 | 15 | 35 |
| Carcass | as available | as available |  |
| Invertebrate (2g) | Live whole animal | 10 | 10 | 20 |
| Black rat  (*Rattus rattus*) | Live whole animal | 15 | NA | 15 |
| Carcass | as available | min 15 | 15 |
| DNA tissue (tail snip) | - | - | 60 (30) individuals |
| Bird | Carcass | as available | as available |  |
| **TOTAL** | **Live whole animal** | **85** | **55** | **140** |
| **TOTAL** | **Carcass** | **-** | **-** | **15** |
| **TOTAL** | **DNA sample** | **-** | **-** | **30** |

**Table 3.** Sample collection schedule for brodifacoum residue on Desecheo Island, March-April 2016.

1. **REPORTING**

The total number of species collected, the sample type of each species (live/carcass), collection date, time, location, collector, and disposition of sample will be provided in a summary report.

* + 1. **Laboratory Residue Analyses**

1. **METHODS (SUMMARY)**

HPLC (high pressure liquid chromatography) will be used to detect and estimate residues of brodifacoum in vertebrate and invertebrate tissues. Typically for larger vertebrate samples, livers will be analyzed; for smaller samples whole-body analysis will be conducted. HPLC provides a concentration (ppm or mg/g) that can be compared and among samples. Analyses will be conducted at the USDA-NWRC laboratory.

1. **REPORTING**

Concentrations of brodifacoum residue assays will be reported in summary tables for the pre- and post-bait application periods and tabulated for concentrations found in each animal species.

1. **Rat Eradication Confirmation Monitoring**
   1. **Genetic Sampling of Rats**

Currently, approximately 130 individual tail samples are archived at Ecogene (New Zealand) collected in 2010 (pre-eradication) from rats across the island. An additional 20 samples were collected in 2013 (post-eradication).

DNA samples will be again collected from both live and dead rats and archived for future use to determine if an eradication failure might be the result of re-emergence (same gene pool) or reinvasion (different gene pool). Samples will be archived at appropriate facilities. At least 30 samples are required for adequate genetic analyses.

Methods for Tail Snip collection

1. Clean rat tail in rubbing alcohol to remove dirt, use gloves.
2. Rinse in water a couple of times to remove excess alcohol before transferring to the vial.
3. Snip c. 1cm of tail tip with scissors and place in vial of Longmire buffer.
4. Buffer must cover tail sample but vial must not be completely full.
5. Place label inside the sample vial with sample details. Internal labels must be included with each sample, in case the external labels are lost. Use white cardstock for label written in pencil.
6. Label outside of vial with an adhesive label.
7. Screw sample vial lids on tightly, and seal in place with Parafilm™.
8. Pack samples in Tupperware container lined and stuffed with paper towels to prevent jostling and provide absorption in the event of a leaky vial. Pad the vials to keep them upright and in place.
   1. **Rat Detection Surveys**

In 2012 on Desecheo, a live rat that had been exposed to bait was captured 23 days after the first bait application and 10 days after the second bait application, in the camp area. In 2011 on Palmyra Atoll, a juvenile rat was captured 28 days after the first application and 18 days after the second application also in the camp area (the eradication was successful). In 2011 on Wake Island, a young black rat was captured 18 days after the first bait application and 9 days after the second bait application, at the Golf Clubhouse (eradication of black rats was successful).

If live rats are detected in between the 1st and 2nd bait applications, no additional bait application is necessary. If live rats are detected more than 7 days after the 2nd bait application, additional hand broadcast or bait stations should be applied in the same area.

While no additional surveys for rats will be implemented before the interim confirmation evaluation (Nov/Dec 2016) and the final eradication confirmation (May 2017), if rats are detected during the biosecurity monitoring activities or opportunistically by other island users, the response actions in the Biosecurity Plan (**Appendix R**) must be implemented.

Two dedicated surveys will be implemented on Desecheo to confirm rat eradication:

* *Interim Survey* - An initial survey to detect rats will be conducted in November/December 2016, after the seasonal wet season.
* *Final Survey* - A final survey to detect rats will be conducted at no sooner than one year post-bait application (c. May 2017) to confirm rat eradication.

The following survey methods will be used for both confirmation surveys:

1. **Detection Stations**

Chew blocks, chew tags, chew sticks, track pads, and tracking tunnels are effective detection tools with which to confirm rodent eradication.

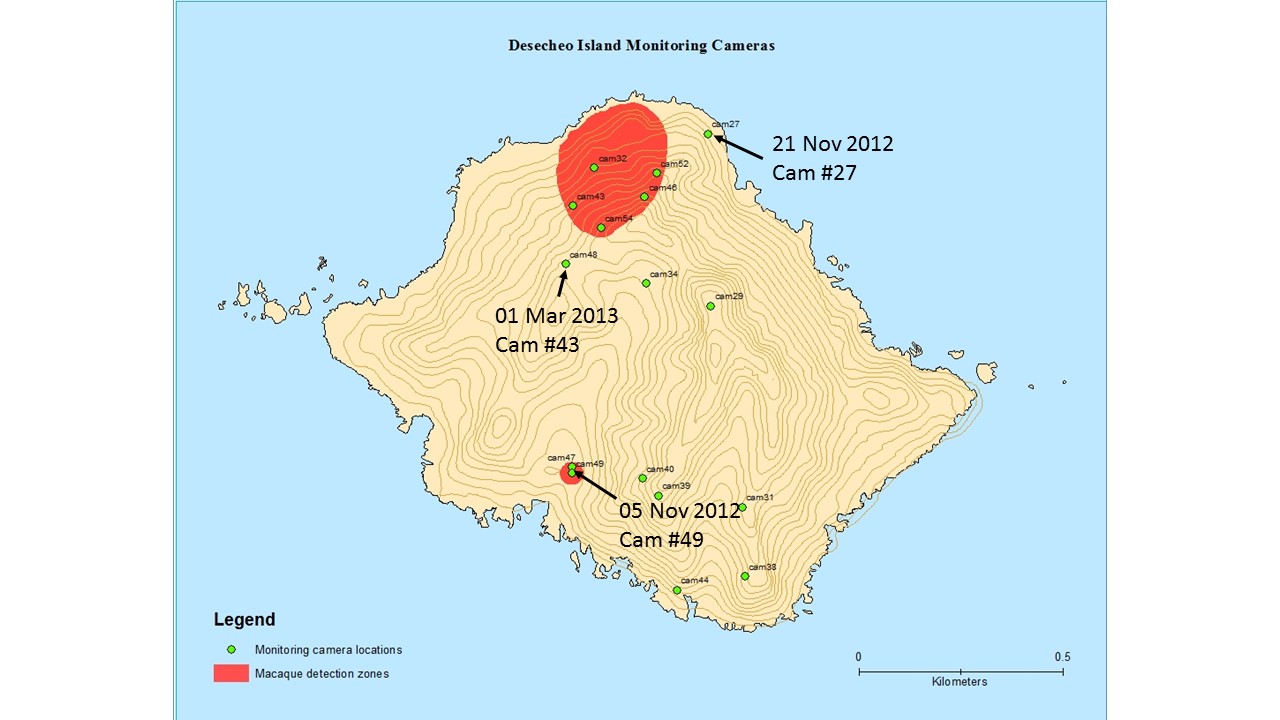
Establishing detection stations

1. Detection stations will be monitored for 1,000 station nights along two transects on the island. Ideally, 100 stations should be deployed over the course of 10 nights, or 200 stations deployed over 5 nights if less time is available.
2. Transects will be located along the ridgeline to the top camp and in Long Valley.
3. Chew block stations will be spaced at 50 meter intervals.
4. Chew blocks will be nailed to trees; trees should be marked with flagging.
5. Every 10th chew block station will have a tracking tunnel stationed in a nearby (within 2m) tree. Tracking tunnels should be secured to a horizontal branch and the tree should be flagged. Tracking tunnels will be monitored for a total of 100 station nights.

Monitoring detection stations

1. Each station will be checked every day.
2. Chew blocks will be assessed for rat incisor marks. Any chew block which shows rat sign will be collected and replaced with a fresh chew block.
3. Chew blocks should be replaced every three days.
4. If the chew block has been cleaned by ants, destroyed by crabs, or otherwise compromised, the chew block should be replaced.
5. If tracking pads have been destroyed by crabs, or otherwise compromised, the trackpad should be replaced.
6. **Motion-sensor Cameras**

Motion-sensor cameras are a cost and time-efficient tool with which to monitor rodent activity. After aerial bait broadcast on Desecheo Island in March 2012, rats were again detected 7 months later (Nov 2012) demonstrating their effectiveness as a confirmation tool (*Fig. 4*). About 25 motion-sensor cameras currently monitoring remnant macaques will be re-programmed to trigger at night so that rat activity can also.



**Figure 4.** Motion sensor camera locations on Desecheo, Dec 04, 2012. Arrows point to the camera locations and dates when rats were first detected after March 2012 aerial bait broadcast: #47, #27, #43.

1. **Conservation Measures**

The goal of the Conservation Measures program for Desecheo Island is to document the long-term ecological responses to the removal of invasive vertebrates (macaques, goats, and rats), and the recovery of endangered and endemic species.

Based on a simplified food web model of the island community, it is anticipated that the removal of macaques, goats, and rats will result in the recovery of the native biological community on Desecheo Island. Specifically, the removal of macaques is expected to result in the recovery of breeding populations of large-bodied seabirds on the island (boobies and frigate birds), as well as have potentially beneficial effects on the reptiles and plant community. The eradication of rats is expected to lead to the recovery of breeding populations of small-bodied seabird species (noddies, terns), as well as potentially beneficial effects on land birds, the reptiles, invertebrates, and the plant community.

Baseline surveys of the island’s seabirds and reptiles were initiated in 2009. Three surveys were completed in 2009 (March, June, December), two surveys in 2010 (March, June), and one survey in each of 2011 (March), 2012 (Feb-April) and 2013 (May-June). All surveys follow the standard protocols established for this program (*University of California Santa Cruz. Standard Monitoring Protocols: Desecheo Island, Puerto Rico, Revised: February 2010*) and (*Island Conservation - Protocols for monitoring Harrisia portoricensis on Desecheo NWR, August 2012).*

During the 2016 rat eradication monitoring period, systematic and opportunistic surveys will be conducted as time and personnel allows including: seabirds, the endangered *Harrisia portoricensis*, vegetation cover, land birds and shorebirds, and the recent invasion of green iguana (*Iguana iguana*). Monitoring techniques used will follow standard protocols (as above), utilize remote camera traps, and new techniques will be tested to address new issues (e.g. green iguana monitoring) or to test improved methods (e.g. reptile surveys).