

United States Department of Agriculture
Animal and Plant Health Inspection Service/Wildlife Services
National Wildlife Research Center

PROTOCOL COVER PAGE

Study Title:	PALMYRA ATOLL RAINFOREST RESTORATION PROJECT: RAT ERADICATION MONITORING PLAN FOR ALTERNATIVES B AND C (AERIAL BROADCAST OF 25W)
NWRC Study Director:	William C. Pitt
Approved NWRC Project:	This study will be conducted under the "Methods and strategies to manage invasive species impacts to agriculture, natural resources, and human health and safety" project of the National Wildlife Research Center

PROTOCOL CLASSIFICATION

1 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection, experiments, or animal studies, and there is generally no commitment of NWRC resources other than personnel time, and activities are not regulated research activities.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> Writing or collaborating on review papers and synthesis reports Student committee participation Analyzing or writing up data collected under operational or other contexts
2 <input type="checkbox"/>	<p>NWRC staff are not involved in study design, data collection or experiments, but the activity involves regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 3 (Description of Activities)</p> <p><input type="checkbox"/> Attach the NWRC or collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval as applicable.</p> <p><input type="checkbox"/> Attach the NWRC Material Transfer Agreement [Standard Form (intellectual property) or Animal/Animal Tissue Transfer Form, as applicable]</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> Training programs requiring the use of animals Providing intellectual property to other organizations for their research purposes (standard Material Transfer Agreement required) Providing animals, tissues or samples to other organizations for their research purposes (Material Transfer Agreement for animal/animal tissue required)
3 <input type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, but the NWRC portion of the study does not include regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input type="checkbox"/> Cover Page <input type="checkbox"/> Part 1 (Signature Page) <input type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input type="checkbox"/> Attach the collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> Collaborating on study design, data analysis, or economic analysis. Minor participation on a regulated study at the collaborating host institution A study that does not include animal use, etc.
4 <input checked="" type="checkbox"/>	<p>NWRC staff actively participate in all or some aspects of the research, and the study involves NWRC facilities and staff, and the study includes regulated research activities*.</p> <p><u>Complete & Submit:</u></p> <p><input checked="" type="checkbox"/> Cover Page <input checked="" type="checkbox"/> Part 1 (Signature Page) <input checked="" type="checkbox"/> Part 2 (Regulatory Considerations) <input checked="" type="checkbox"/> Part 4 (full NWRC Study Protocol)</p> <p><input checked="" type="checkbox"/> Complete and attach any appendices required under Part 2 including collaborating institution's appropriate regulated documentation (IACUC, Biosafety, NEPA, ESA) & approval if necessary.</p>	<p><u>Examples:</u></p> <ul style="list-style-type: none"> A typical NWRC led study Major NWRC staff participation in regulated activity Study takes place on NWRC facilities

* Regulated research activities include the use of animals, controlled materials, microbiological/biohazardous agents, test material/device; impacts historical resources, the environment or endangered species. See the Animal Use Appendix for a definition of "animal" and "animal use".

PART ONE: SIGNATURE PAGE

Study Director:

William C. Pitt

Date:

May 12, 2011

Position (check one):

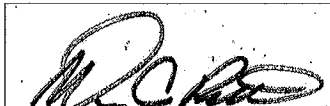
☐ Biologist/Chemist/Technician
Supervisor signature required:

Date _____

☐ Res. Scientist☐ Proj. Leader☐ Research Scientist☒ Project Leader☐ Visiting Scientist: NWRC Representative/Contact: _____☐ Student: NWRC Representative/Contact: _____

Concur:

NWRC Research Project Leader

Date 20 May 11

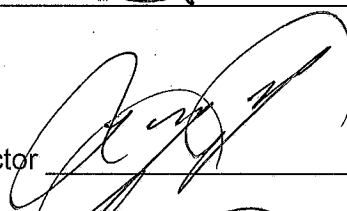
Review and Processing:

QAU:

Date 5/26/11

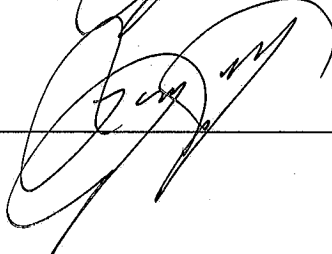
Concur:

NWRC Assistant Director

Date 5/26/11

Approved:

NWRC Director

Date 5/26/11

Note: Additional approvals are located in the attached appendices.

NO	YES	Item
Animal Use		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study include the use of animals? An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals. <input checked="" type="checkbox"/> NWRC is responsible for all or part of live animal phase; attach NWRC Animal Use Appendix <input type="checkbox"/> Collaborating institution is responsible for all or part of live animal phase; attach IACUC protocol & approval <input type="checkbox"/> Animal samples will be incidentally collected and received from existing WS operations. NWRC personnel are <u>not</u> involved in collection or design of the operation.
Microbiological/Biohazardous Materials		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will any Microbiological/Biohazardous Materials be used? If yes, please complete and attach Microbiological/Biohazardous Materials Use Appendix .
Permits		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will permits be required (e.g., collecting, marking, banding, or sampling permit)? If yes, list all pertinent the State and Federal animal use/scientific collection permits, Migratory Bird Treaty Act or Endangered Species Act permits, Animal Health certificate, chemical experimental use permits, agreements, permit for controlled organisms, etc. Include all required permit numbers and approval dates. Collection permit Importation permit USFWS Supplemental Use Permit Application Pending USFWS Import Permit Notification complete/Permit process on site USDA Soil Transit Permit Application Pending
National Environmental Policy Act (NEPA) and Endangered Species Act (ESA)		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will study result in mortality, removal, live-capture/release, harassment of animals from/in the wild, impact their natural habitat (including application of test materials/devices) or impact non-target animal populations (i.e., could or may result in their death or serious injury)? If yes, complete the NEPA & ESA Appendix .
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Could study result in the disturbance, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles? If yes, complete the NEPA & ESA Appendix . Contact QA/NEPA staff for ESA or eagle incidental take requirements.
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does this study involve interstate transport of live wildlife? If yes, contact QA/NEPA staff for Lacey Act requirements.
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will this involve the international import or export of animal tissues or specimens? If yes, add permit information above.
Regulatory Standard and Test Guidelines		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Does this study have the potential to be part of a product registration data submission? If yes, date of consult with Registration Manager: ____5/12/2011____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any regulatory standard? If yes please check: <input type="checkbox"/> CFR Title 40, Part 160: Good Laboratory Practice Standards (EPA FIFRA) <input type="checkbox"/> Other:_____
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study be conducted under any testing guideline (e.g., EPA Testing Guidelines)? If yes, please list the guideline:
Test, Control and Reference Material/Devices		

<input checked="" type="checkbox"/>	<input type="checkbox"/>	Will this study include the testing of any article, material or device? If yes, attach the Test, Control and Reference Material/Devices Formulation and Use Appendix . Please indicate if otherwise described in the protocol.
Historical Resources		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve any major ground disturbance, loud noises, or other activity that has the potential to adversely affect historic resources (e.g. placing exclusion devices/noises around historic places)? If yes, provide information and consult with the State Historic Preservation Office.
Material Transfer Agreement		
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Does the research involve the transfer of materials (intellectual property, controlled materials, animals, animal tissues, etc.) to another facility? If yes, complete the appropriate Material Transfer Agreement .
Analytical Chemistry		
<input type="checkbox"/>	<input checked="" type="checkbox"/>	Will any chemical analysis be required of the NWRC Analytical Chemistry Project (ACP)? If yes, attach Analytical Chemistry Appendix .

PART FOUR: FULL NWRC STUDY PROTOCOL

1. Key Personnel

Name	Organization	Role in Study
Study Director		
William C. Pitt, PhD.	USDA/APHIS/NWRC	Study Director
Other Investigators, Collaborators, Cooperators, and Consultants		
Kathleen Hayes	USDA/APHIS/NWRC, Hawaii	Monitoring Team, Technician
Thomas McAuliffe	USDA/APHIS/NWRC, Hawaii	Monitoring Team, Technician
Are Berentsen	USDA/APHIS/NWRC, Colorado	Monitoring Team Lead, Biologist
Amanda Meyer, PhD.	U.S. Fish and Wildlife Service	Monitoring Team, Biologist, Refuge Manager
Stacie Hathaway	U.S. Geological Survey	Monitoring Team, Biologist
Alex Wegmann, PhD.	Island Conservation	Project Manager for Rodenticide Application
Evelyn Wight Mark Fox	The Nature Conservancy	Communications Manager Director of External Affairs
Beth Flint	U.S. Fish and Wildlife Service	Certified Applicator

2. Testing Facilities

Name	Address	Role in Study
USDA/APHIS/NWRC	4101 LaPorte Ave., Ft. Collins, CO 80521	Testing for traces of rodenticide residue in non-target specimens

3. Sponsor

Name	Address	Contract No.
Island Conservation Contact: Alex Wegmann	Center for Ocean Health 100 Shaffer Rd. Santa Cruz, CA 95060	Pending

4. Schedule

Proposed Experimental Start Date: June 1, 2011
Proposed Experimental Termination Date: July 5, 2011
Proposed Study Completion/Archive Date: May 31, 2012

5. Background and Justification

Palmyra Atoll (Palmyra), co-managed by the US Fish & Wildlife Service and The Nature Conservancy, is an important center of biodiversity and species abundance in the Central Pacific region. No baseline record of Palmyra's terrestrial ecosystem prior to the 19th century exists; however, it is possible that Polynesian voyagers altered Palmyra's environment prior to European exploration of the Pacific by introducing coconut palms. Anthropogenic impacts accelerated during and after WWII with introductions of other plants, terrestrial arthropods, and mammals. Rats were likely introduced to the atoll during this period.

Introduced non-native species are a leading cause of extinctions in island communities worldwide. Removing rats from Palmyra will result in biodiversity benefits for seabirds, plants, terrestrial invertebrates, and other components of the atoll's terrestrial ecosystem; however, the removal process is complicated and could cause short- or even long-term damage to some of Palmyra's animal populations. If the removal of rats from Palmyra does cause harm to local individual animals or animal populations, we anticipate that this impact will be short-lived and overshadowed by the large restoration benefit achieved through the removal of rats.

In June of 2011, the Palmyra Atoll Rainforest Restoration Project (PARRP) partnership will treat the entire atoll with rodenticide to remove the alien rat (*Rattus rattus*) population. The eradication will involve both aerial and ground application of bait containing the second generation anticoagulant rodenticide brodifacoum at a concentration of 25 ppm (Brodifacoum 25 W Conservation). All emergent land areas will be treated, and measures will be employed to prevent intentional and minimize accidental bait drift into the marine environment.

Bait will be applied to the atoll according to a strategic plan that will minimize the risk of bait drift into the marine environment while ensuring that a sufficient amount of bait is delivered to every potential rat territory. Two applications of bait over the entire atoll will be conducted during the eradication, and each application will employ three methods of aerial broadcast baiting (full swath - 40m, directional swath - 20m, and narrow swath - 10 m), and hand baiting. Bait will be slung into the crowns of coconut palms (*Cocos nucifera*) that overhang near-shore waters as this habitat will not be baited during the aerial application, and bait stations will be

placed and maintained around the camp area and on select small islands that are thought to be rat-free.

The operation is scheduled to coincide with the summer migration of shorebird species that are at risk of exposure to the rodenticide. The implementation team will include 4-5 personnel who are dedicated to monitoring the parameters of the eradication, such as bait application rate and bait fate, and environmental factors, such as rodenticide residue in water and non-target species exposure to the rodenticide. All sampling devices, including location markers, such as flagging tape, will be recovered once the monitoring goals have been met.

The monitoring actions described in this plan will occur prior to, during, and after the implementation of the eradication. This plan builds on prior studies of Palmyra's eradication environment (Howald et al. 2004, Buckelew et al. 2005, USDA-APHIS 2006, Wegmann et al. 2008, Alifano and Wegmann 2010, Alifano et al. 2010), rodent eradication studies conducted in similar environments (Wegmann et al. 2006, Wegmann et al. 2007, Wegmann 2008), and decades of research on the management of invasive species (Witmer et al. 2007). The monitoring activities, number of samples that will be collected will be required for each sampling activity is presented in Table 1.

This conservation action, including the monitoring component, will be a cooperative effort by the US Department of Agriculture, US Fish and Wildlife Service, The Nature Conservancy, and Island Conservation.

6. Related Protocols

There are no existing protocols related to this study.

7. Assurance of Non-Duplication of Studies

This project will be the highest density (bait weight/ hectare) aerial broadcast of rodenticide on record. While similar to other aerial broadcasts, it is unique due to its very large scale of application and location on Palmyra atoll.

8. Objective/Hypotheses

1. Verification of the application rate on the ground and in the coconut palm canopy

2. Document adverse impacts, or lack thereof, to Palmyra's biota caused by the rat removal action.
3. Document short term negative impacts, or lack thereof, to Palmyra's biota caused by the rat removal action
4. Establish clear pathways by which non-target species could be exposed to the rodenticide employed to remove rats from Palmyra, and measure rodenticide exposure levels for key non-target species
5. Document that the rat removal action has successfully removed all rats from Palmyra.

9. Methods/Procedures

A. Verification of Target Application Rate:

1. Terrestrial Bait Application Rate

- a. **Personnel Requirements:** 4 people (2 teams of 2 people)
- b. **Equipment Requirements:** Handheld GPS units (2), PVC Hoops (4), Sharpies (4), snack size re-sealable bags ,re-sealable Ziplock freezer bags, Data Collection Forms, field notebook, pens, pencils
- c. **Form:** Terrestrial Bait Application Rate Verification
- d. **Methodology**
 - i. Thirteen locations on Palmyra Atoll will be sampled for aerial baiting rate. Baiting will occur over two days (per application period) (**Figure 1**).
 - ii. Prior to aerial drop, 15 locations will be randomly chosen at each of the designated collection sites (a total of 105 individual sample collection points).
 - iii. The crew will be split into teams of two, each crew member will carry one PVC hoop, 1 box sandwich Ziplocks bags, 10 Freezer Ziplocks, 2 sharpies, field first aid kit, data collection forms, field notebook, and one backpack.
 - iv. Crews will operate as pairs, but each crew member will collect a unique set of bait samples.
 - v. Following as closely behind the helicopter as safely possible, but no more than one half-hour after an area was baited, crews will collect bait samples in the following manner.
 1. Each crew member will randomly select 7 or 8 locations to sample within the sampling area.
 2. Facing north, crew members will drop 1 PVC hoop dropped from shoulder height allowing the hoop to settle on the soil surface. If the PVC hoop hangs up on vegetation, it may be nudged so that

it falls to and rests on the ground.

3. All bait pellets (or fractions of a pellet) should be removed from the area within the PVC hoop and placed inside a labeled sandwich size Ziplock bag. Bait pellets should be considered inside the PVC hoop ring if any portion of the pellet is inside the area bordered by the inside of the PVC hoop tube (**Figure 2**).
 - a. Ziplock bags should be labeled with the following information (with permanent ink, sharpie):
 - i. QA No.
 - ii. Collection Date
 - iii. Crew member Initials
 - iv. Collection Site
 - v. Sample number
4. Sample collection information should be entered on to the 'Terrestrial Bait Application Rate Verification' form.
5. Properly labeled and sealed bags should be placed in a Ziplock Freezer bag labeled with the following information:
 - i. QA No.
 - ii. Collection Date
 - iii. Crew member (s)
 - iv. Collection Site
 - v. Number of Samples in the freezer bag
- vi. When all samples are collected at a collection site, as a team, both crew members should proceed to the next collection site and repeat the sample collection operation.
- vii. When all samples have been collected for the day, the crew should return to camp and weigh each bag using a laboratory balance (0.1g accuracy) and record the weight of the Terrestrial Bait Application Rate Verification form.

Figure 1. A map of the thirteen sampling locations that will be used to measure the bait application rate on the ground during each of the two aerial broadcasts that will occur during the Palmyra Atoll rat



eradication.

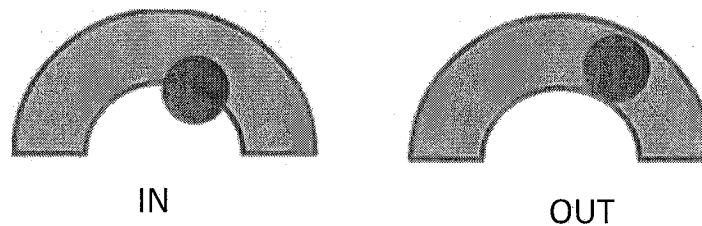


Figure 2. Diagram of how to determine if a bait pellet is inside or outside a PVC hoop collection area.

2. Bait Drift into Aquatic Habitats

- a. **Personnel Requirements:** 2 people – plot construction
1-2 people – Bait collection
- b. **Equipment Requirements:** Handheld GPS units (2), PVC Hoops (154), Sharpies (4), snack size re-sealable bags, re-sealable Ziplock Freezer bags, Data Collection Forms, Field notebook, pens and pencils.
- c. **Form:** Bait Drift Form
- d. **Methodology**
 - i. 10 locations on Palmyra Atoll will be sampled for bait drift into aquatic environments following aerial baiting operations. GPS locations will be recorded for the location of each grid. Aerial baiting will occur over two days (per application period) (**Figure 3**).

- ii. Prior to aerial drop, semi-permanent sampling grids should be established at each of the 10 collection sites. Each sampling grid should be constructed as follows. Hoops should be held in place using 2 or three stakes if the substrate permits or using 2 strings tied to rocks (**Figure 4**).
- iii. Aquatic sampling areas are adjacent to areas designated for terrestrial bait sampling. If tides are appropriate, the person sampling the aquatic grids could conduct their sampling effort in the same areas as the terrestrial crews are working. This would address safety considerations for people working alone.
- iv. The technician sampling these grids will carry 1 box sandwich Ziplocks bags, 20 Freezer Ziplocks, 2 sharpies, field first aid kit, data collection forms, field notebook, and one backpack.
- v. Following as closely behind the helicopter as safely possible and during the tide cycle when water has receded and hoops are lying on the tidal flat, the technician will collect bait samples in the following manner.
 1. All bait pellets (or fractions of a pellet) should be removed from the area within the PVC hoop and placed inside a labeled sandwich size Ziplock bag. Bait pellets should be considered inside the PVC hoop ring if any portion of the pellet is inside the area bordered by the inside of the PVC hoop tube (**Figure 2**).
 - a. Re-sealable bags should be labeled with the following information (with permanent ink, sharpie):
 - i. QA No.
 - ii. Collection Date
 - iii. Crew member Initials
 - iv. Collection Site
 - v. Hoop location in grid (A1, A2, . . .)
 2. Sample collection information should be entered on to the 'Bait Drift' form.
 3. Properly labeled and sealed baggies should be placed in a Ziplock Freezer bag labeled with the following information:
 - i. QA No.
 - ii. Collection Date
 - iii. Crew member (s)
 - iv. Collection Site
 - v. Number of Samples in the freezer bag
- vi. When all samples are collected at a collection site, the technician should proceed to the next collection site and repeat the sample collection operation.
- vii. When all samples have been collected for the day, the crew should return to camp and weigh each baggie using a laboratory balance (0.1g accuracy) and record the contents weight on the Bait Drift Form.

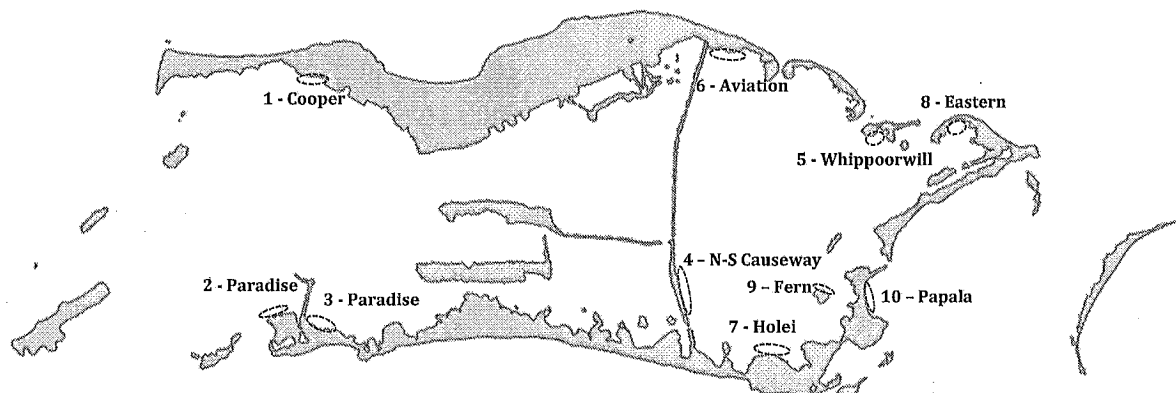


Figure 3. A map of the ten sampling locations that will be used to measure the rate at which bait drifts into the near-shore marine environment during each of the two aerial broadcasts that will occur during the Palmyra Atoll rat eradication.

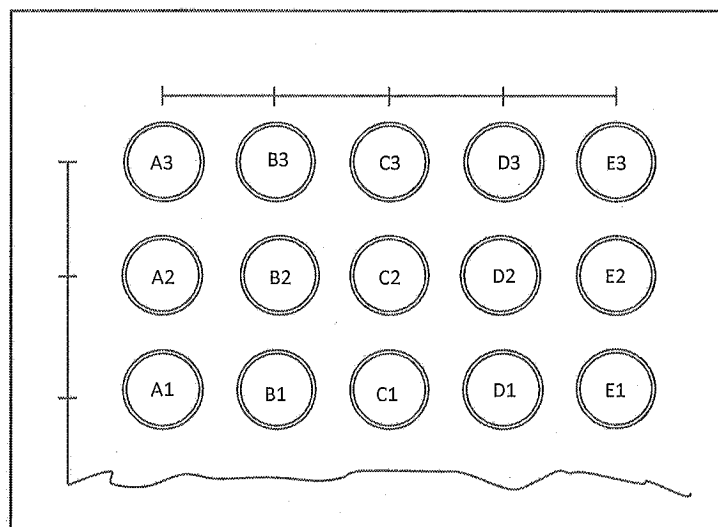


Figure 4. Diagram of how PVC hoops should be laid out in the tidal flats to assess bait drift.

B. Bait Fate Monitoring:

1. Bait Fate on Ground

- a. **Personnel Requirements:** 2 people – plot construction, 2 people – data collection
- b. **Equipment Requirements:** Handheld GPS units (2), GPS Batteries, PVC hoops (10), Sharpies(4), 25W bait (1kg), Gloves, Data Collection Forms, Field Notebook

c. **Form:** Ground Bait Fate Assessment Form

d. **Methodology**

- i. PVC sampling hoops will be fixed in place within representative patches of Palmyra's four main terrestrial habitat types: Coconut Palm Forest, *Scaevola/Heliotropium* shrubland, *Pisonia* forest, and *Lepturus* meadow. The four study patches will be located on Cooper Island (**Figure 5**).
- ii. Ten sampling hoops will be randomly placed in each study area and GPS way points recorded for each hoop. Directly following aerial treatment of the surrounding area, pellets will be added to or removed from the sampling hoops so that the bait density within the hoop equals the target application rate (5 pellets/ hoop).
- iii. All sampling hoop within each study patch will be visited every day until all pellets are gone from within the hoop.
- iv. Observations taken each day will include the number of pellets remaining and the general pellet condition. Pellet condition will be assessed as follows
 1. Bait hard, intact, whole
 2. Bait hard, intact, partially gone
 3. Bait soft, intact, whole
 4. Bait soft, intact, partially gone
 5. Bait mushy, disintegrated
 6. Bait dry, disintegrated
 7. Bait pellet gone
- v. All data should be entered on to the Ground Assessment form.

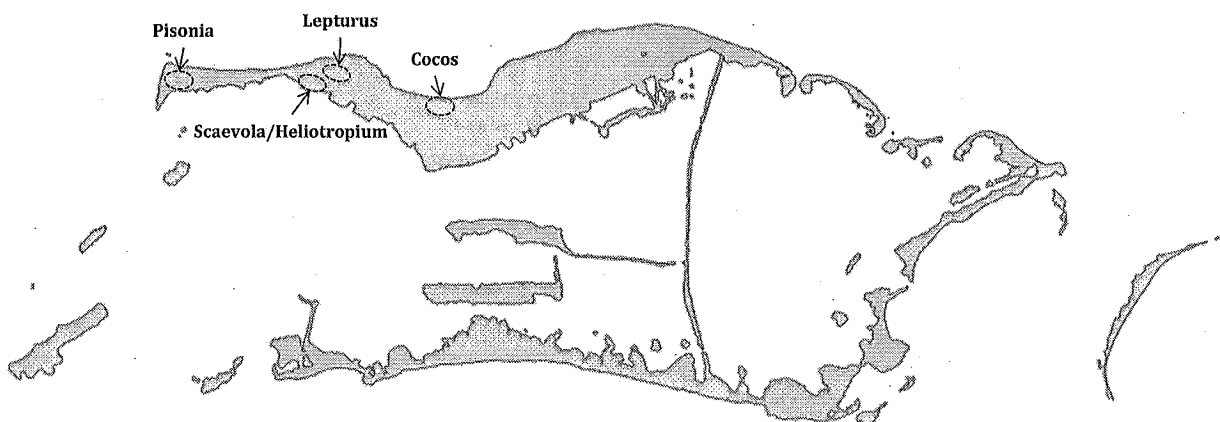


Figure 5. A map of the four sampling locations that will be used to measure the fate of bait pellets after each of the two aerial broadcasts that will occur during the Palmyra Atoll rat eradication.

2. Bait Fate in Canopy

- a. **Personnel Requirements:** 2 people – plot construction, 2 people-sampling

- b. **Equipment Requirements:** Handheld GPS units (2), 40 foot ladder, compass (2), Sharpies(4), Data Collection Forms, Field Notebook
- c. **Form:** Canopy Bait Fate Assessment Form
- d. **Methodology**
 - i. Bait fate in the canopy will be assessed in three locations, all located on Cooper Island. The locations are 1) West Lagoon immediately west of the camp area, 2) North Beach, and 3) east of the northeast end of the runway (**Figure 6**).
 - ii. A total of 15 coconut palms within these three areas that can be easily accessed with a 40' extension ladder will be selected prior to the first bait application.
 - iii. Within 4 hours of aerial bait application, each palm will be checked for the presence of bait pellets.
 - iv. During this initial assessment, the location of five bait pellets (the bait pellets will be found in the tree or placed there if less than five are found) will be indicated by using a permanent marker to draw an arrow that points to the pellet on an adjacent frond. The location will also be noted on the diagram on the Canopy Bait Fate Assessment form.
 - v. Each of the five study pellets in each study tree will be given a unique, two digit identifier ("1-1" will mean that this is pellet 1 in tree 1) and checked daily until the pellet disappears. While the pellets remain in the crowns, pellet fate will be recorded according to the following bait pellet condition scale:
 - 1. Bait hard, intact, whole
 - 2. Bait hard, intact, partially gone
 - 3. Bait soft, intact, whole
 - 4. Bait soft, intact, partially gone
 - 5. Bait mushy, disintegrated
 - 6. Bait dry, disintegrated
 - 7. Bait pellet gone
 - vi. All data should be entered on to the Canopy Bait Fate Assessment form.

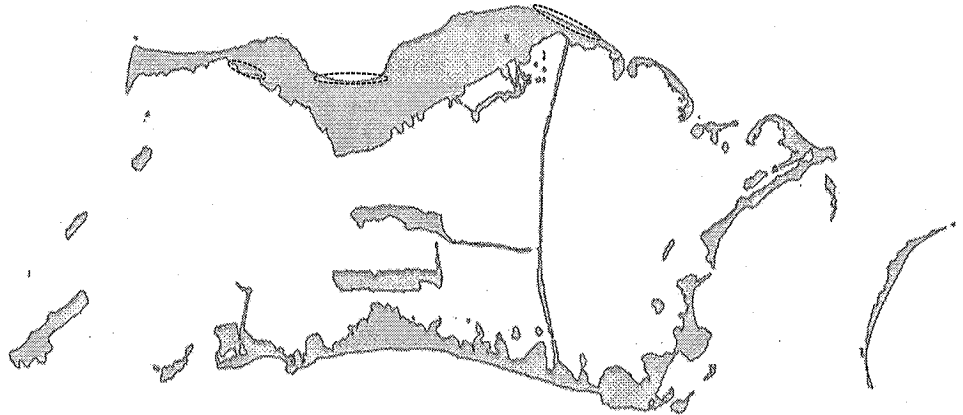


Figure 6. A map of the three sampling locations that will be used to measure the fate of bait pellets lodged in the crowns of palm trees after each of the two aerial broadcasts that will occur during the Palmyra Atoll rat eradication.

3. Bait Caching and Fate in Crab Burrows

- a. **Personnel Requirements:** 2 people – plot construction
1 people – data collection
- b. **Equipment Requirements:** Handheld GPS units (2), GPS Batteries, PVC hoops (10), Sharpies(4), placebo bait (1kg), Gloves, Data Collection Forms, Field Notebook
- c. **Form:** Bait Caching and Fate Assessment Form
- d. **Methodology**
 - i. During the 7 days prior to the aerial application of toxic bait, caching behavior and rate by *Cardisoma* and fate of the bait cached in burrows will be assessed using placebo (non-toxic) bait.
 - ii. Ten active *Cardisoma* will be located and marked with flagging and located using a handheld GPS unit. Burrow activity will be assessed by the presence of crabs in burrow or clear sign that burrows have been utilized in the recent past. The presence of cobwebs or untracked sand in the burrow is evidence it is not being used. Burrows should be shallow enough that the bottom of the burrow can be easily observed with a strong flashlight and the pellets can be poked with a narrow rod.
 - iii. Using a 1 meter string or rod, mark a 1m circle around the burrow opening by using the heel of a shoe or stick to carve a furrow in the sand.
 - iv. Clear all debris out of the area inside the marked circle and randomly place 15 pellets within the circle. Note on Bait Caching and Fate

Assessment from the approximate locations of the bait pellets within the circle and condition of the pellets.

- v. Monitor the fate of the pellets at 24 hour intervals.
- vi. Records noted at each visit should include any observations of any animals or invertebrates interacting with the bait. Number of baits remaining on the soil surface, number of pellets cached in the burrow, and condition of the pellets in the burrow.
- vii. Pellet condition should be assessed using the following classification system.
 1. Bait hard, intact, whole
 2. Bait hard, intact, partially gone
 3. Bait soft, intact, whole
 4. Bait soft, intact, partially gone
 5. Bait mushy, disintegrated
 6. Bait dry, disintegrated
 7. Bait pellet gone

C. Non-target Sampling:

1. Shorebird count and carcass sampling

- a. **Personnel Requirements:** 1 personnel for shorebird count, 2 personnel for carcass search
- b. **Equipment Requirements:** Handheld GPS units (2), gloves, Ziplock bags, Sharpies, Field Notebook, Pens and Pencils.
- c. **Form:** Non-target species sampling form
- d. **Methodology:**
 - i. To document harm caused to non-target organisms due to exposure to rodenticide, a team of 2 people will conduct directed carcass searches, and will sample terrestrial organisms for evidence of exposure. In addition, all carcasses located opportunistically during all eradication operations will be collected for tissue collection for residue analysis. Locations of carcasses will be recorded using handheld GPS units.
 - ii. Organized searches will occur along set transects located at the North Beach and runway roost/loafing sites during and after the eradication. In addition, carcass searches will be performed on Dadu Island during shore bird counts and baiting operations. Because of the lack of crab foraging, bird carcasses should persist longer on Dadu Island.
 - iii. Starting three days prior to the first bait application and continuing until 10 days after the second bait application, organized carcass searches will occur on the runway and on North Beach – both are prominent, easily accessed shorebird roosting sites. In addition to this search effort, an all atoll shorebird count and carcass search (**Figure 7, 8**) will occur at the following intervals after the first bait application: days 5, 8, 16, and 19. Furthermore, all project staff will be instructed on proper collection and tagging protocols should they come across a carcass during other project activities – this will allow for opportunistic documentation of non-

target species response to the eradication action. All dead or moribund target and non-target individuals found during and up to 2 years after the operation will be collected and stored in receptacle and used for tissue collection. In the case that euthanasia is needed for the moribund individuals, an overdose of anesthetic will be used. The resulting samples will be labeled for lab-based detection and quantification of rodenticide residue.

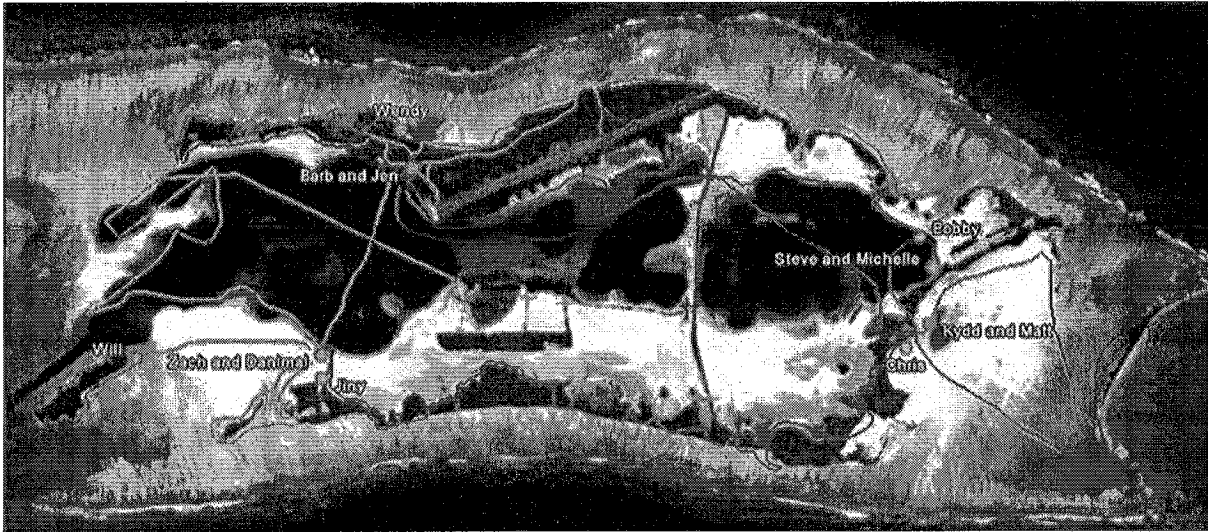


Figure 7. A depiction of the routes followed during an "All-Atoll" shorebird count and carcass search.

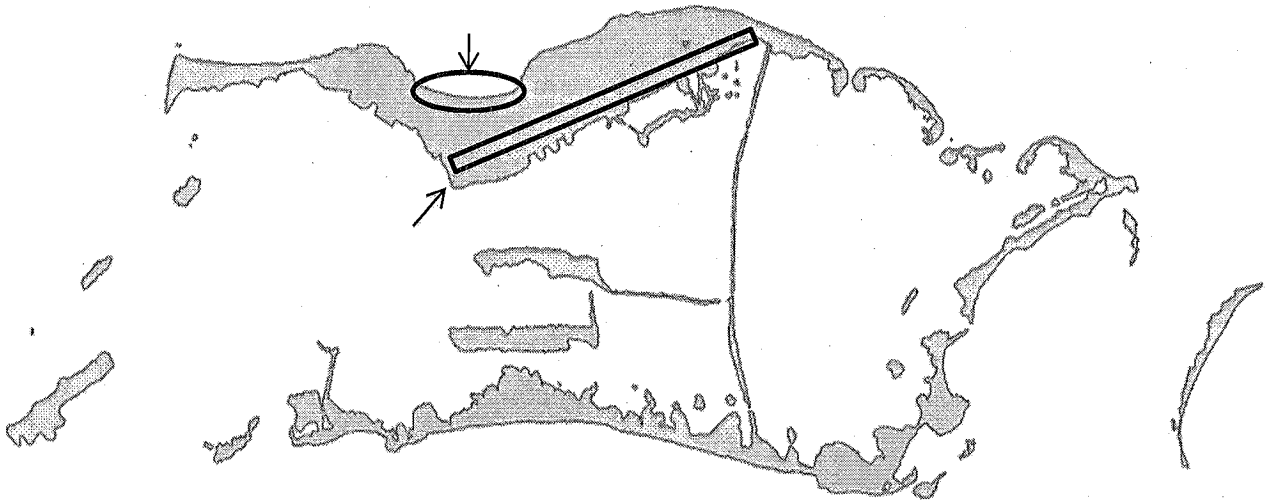


Figure 8. The locations where daily carcass searching will occur during the Palmyra Atoll rat eradication.

2. Gecko sampling

- a. **Personnel Requirements:** 2 personnel
- b. **Equipment Requirements:** Handheld GPS units (2), gloves, Ziplock bags, Sharpies, Field notebook, pens and pencils, pithing rod.
- c. **Form:** Non-target species sampling form

d. Methodology:

- i. Twenty two samples consisting of up to 5 individuals per sample (110 individuals) will be collected from Cooper Island at five intervals according to the following schedule:

1-7 days prior to 1st bait application: 2 samples (10 individuals)

1-4 days after 1st bait application: 5 samples (25 individuals)

9-11 days after 1st bait application: 5 samples (25 individuals)

21-24 days after 1st bait application (~7-8 days after 2nd application): 5 samples (25 individuals)

56 days after 1st bait application (~42 days after 2nd application): 5 samples (25 individuals)

- ii. Geckos will be opportunistically collected using hand-capture primarily, and trapped using commercially available insect and rodent glue traps as a secondary method. Glue traps will be set in the evening and checked within 24 hours of being set, and subsequently within 24 hours of the last trap check. A commercially available solvent, (Goof Off,® Memphis, TN) will be used to remove trapped geckos from traps. Non-target vertebrates will be released or euthanized using cervical dislocation or overdose of isoflurane gas if they are severely injured from glue board trapping. Geckos will be anesthetized using an inhalant (isoflurane gas), then euthanized by decapitation, then pithed, and placed in individually labeled 4X6 inch plastic bags and placed in the freezer. When each sampling interval is complete, sample bags containing individual geckos will be placed in a large (9X26 inch) plastic bag and grouped by sampling interval and returned to the freezer. Samples will be shipped frozen to USDA/APHIS/WS/NWRC field station in Hilo, Hawai'i and then on to the NWRC in Fort Collins for analysis.

3. Ant sampling

- a. **Personnel Requirements:** 2 personnel
- b. **Equipment Requirements:** Handheld GPS units (2), "pitfall traps" (20), gloves, sieve, paper towels, dish soap, sugar cubes, collection vials, sharpies, field notebook, pens and pencils.
- c. **Form:** Non-target species sampling form
- d. **Methodology:**
 - i. Twenty two pooled samples consisting of up to 5g per pooled sample will be collected from Cooper Island at five intervals according to the following schedule:

1-7 days prior to 1st bait application: 2 samples

1-4 days after 1st bait application: 5 samples

9-11 days after 1st bait application: 5 samples

21-24 days after 1st bait application (~7-8 days after 2nd application): 5 samples

56 days after 1st bait application (~42 days after 2nd application): 5 samples

- ii. Ants will be collected opportunistically using pitfall traps. Twenty, one quart Tupperware bowls will be filled with water, two sugar cubes and a few drops of commercial dish soap to reduce water surface tension. Sampling will take place on Cooper Island in areas known to have ants. Pit traps will be checked before sunset on trapping days. Bowls containing ants will be poured through a common kitchen sieve, allowed to dry on paper towel and placed in a 20 ml HDPE scintillation vial. Tare weights of empty vials will be obtained and the sample weight will be calculated by difference. Individual sample vials will be labeled according to sample period and frozen. Samples will be shipped frozen to USDA/APHIS/WS/NWRC field station in Hilo, Hawai'i and then on to the NWRC in Fort Collins for analysis.

4. Cockroaches sampling

- a. **Personnel Requirements:** 2 personnel
- b. **Equipment Requirements:** Sticky traps, collection vials, notebook, pens and pencils, Ziplock bags (9 x 26 inch size)
- c. **Form:** Non-target species sampling form
- d. **Methodology:**
 - i. Twenty two samples consisting of up to 5 individuals per sample (110 individuals) will be collected from Cooper Island at five intervals according to the following schedule:

1-7 days prior to 1st bait application: 2 samples (10 individuals)

1-4 days after 1st bait application: 5 samples (25 individuals)

9-11 days after 1st bait application: 5 samples (25 individuals)

21-24 days after 1st bait application (~7-8 days after 2nd application): 5 samples (25 individuals)

56 days after 1st bait application (~42 days after 2nd application): 5 samples (25 individuals)

- ii. Cockroaches will be opportunistically collected using commercially available traps (Hoyhoy cockroach trap). Traps will be checked every 24 hours during the sampling interval. Cockroaches will be removed from the trap and placed in a labeled 20 ml scintillation vial and frozen. When each sampling interval is complete, vials of cockroaches will be pooled by sampling interval and placed in a large (9X26 inch) plastic bag and returned to the freezer. Samples will be shipped frozen to USDA/APHIS/WS/NWRC field station in Hilo, Hawai'i and then on to the NWRC in Fort Collins for analysis.

5. Fish sampling (Black spot sergeant (*Abudefduf sordidus*))

- a. **Personnel Requirements:** 2 personnel
- b. **Equipment Requirements:** spear guns (2), nets, collection receptacles, plastic bags (9 x 13 inch), sharpies, field notebook, pens and pencils, pithing rod.
- c. **Form:** Non-target species sampling form
- d. **Methodology:**
 - i. Samples comprising three black spot sergeant fish will be collected from each of ten difference locations no more than 7 days before the first application and 1-2 days after the second application (60 fish total). The primary sampling method will be by spear-gun. Hoop/seine nets may be erected as a secondary capture method. In the event nets are used, nets will be set up in shallow water (1m deep) during the morning high tide and checked as the tide recedes. Fish removed from nets will be stunned, and then euthanized by decapitation followed by pithing in concordance with the AVMA guidelines for euthanasia of fish. Non-target captures will be released.
 - ii. Individual fish will be placed in a 9x13 inch plastic bag, then pooled into a larger plastic bag labeled by location and time period. Samples will be shipped frozen to USDA/APHIS/WS/NWRC field station in Hilo, Hawai'i and then on to the NWRC in Fort Collins for analysis.

6. Fiddler Crab Sampling

- a. **Personnel Requirements:** 2 personnel
- b. **Equipment Requirements:** Shovels (2), Hand towels, plastic bags, sharpies, notebook, pens and pencils
- c. **Form:** Non-target species sampling form
- d. **Methodology:**
 - i. Twenty two samples consisting of up to 5 individual per sample will be collected from Cooper Island at five intervals according to the following schedule:

1-7 days prior to 1st bait application: 2 samples (10 individuals)

1-4 days after 1st bait application: 5 samples (25 individuals)

9-11 days after 1st bait application: 5 samples (25 individuals)

21-24 days after 1st bait application (~7-8 days after 2nd application): 5 samples (25 individuals)

56 days after 1st bait application (~42 days after 2nd application): 5 samples (25 individuals)
 - ii. Fiddler crabs will be collected from mudflats during low tide. Crab holes will be located and samples will be collected by excavating burrows with a shovel or hand trowel. Individual samples (up to 5 individuals per sample) will be placed in a single, labeled plastic bag and frozen. Samples will be shipped frozen to USDA/APHIS/WS/NWRC field station in Hilo, Hawai'i and then on to the NWRC in Fort Collins for analysis.

7. Hermit Crab Sampling

- a. **Personnel Requirements:** 2 personnel
- b. **Equipment Requirements:** Hand towels, plastic bags, sharpies, notebook, pens and pencils
- c. **Form:** Non-target species sampling form
- d. **Methodology:**
 - i. Twenty two samples consisting of up to 5 individual per sample will be collected from Cooper Island at five intervals according to the following schedule:

1-7 days prior to 1st bait application: 2 samples (10 individuals)

1-4 days after 1st bait application: 5 samples (25 individuals)

11-14 days after 1st bait application: 5 samples (25 individuals)

21-24 days after 1st bait application (~7-8 days after 2nd application): 5 samples (25 individuals)

56 days after 1st bait application (~42 days after 2nd application): 5 samples (25 individuals)

- ii. Hermit crabs will be picked up by hand and collected randomly from the ground. Individual samples (up to 5 individuals per sample) will be placed in a single, labeled plastic bag and frozen. Samples will be shipped frozen to USDA/APHIS/WS/NWRC field station in Hilo, Hawai'i and then on to the NWRC in Fort Collins for analysis.

D. Environmental Sampling:

1. Water Sampling

- a. **Personnel Requirements:** 1 personnel
- b. **Equipment Requirements:** gloves, 1-liter water bottles, sharpies, foam coolers (36-quart), gel ice packs, notebook, pens and pencils
- c. **Form:** Location and sample number recorded in study notebook
- d. **Methodology:**
 - i. Twelve one-liter water samples will be collected at the following locations:

One sample from each of three sites outside the lagoon (western terrace).

One sample from each of six sites adjacent to baited areas in deep ($\geq 1\text{m}$) water.

One sample from the brackish eel pond adjacent to Cooper Island.

One sample from the runway pond.

One sample from the groundwater well on Eastern Island.

- ii. Sampling will be conducted at four intervals, for a total of 48 samples:

One complete set of samples ≤ 7 days prior to the first application

One complete set of samples 1 day after the first application

One complete set of samples 1 day after the second application

One complete set of samples 7-10 days after the second application

- iii. One person will be responsible for all water sample collection. The collector will wear protective gloves during all water sampling. Chemically cleaned 1-liter bottles will be used. After removing the top, labeled collection bottles will be submerged in water until the top is just below the water surface, allowing the bottle to fill completely and the top will be secured. Samples will be refrigerated after collection. Water samples will be packed in commercially available 36 quart coolers with protective foam padding and kept cold with gel packs during shipment to USDA/APHIS/WS/NWRC field station in Hilo, Hawai'i and then on to the NWRC in Fort Collins for analysis.

2. Soil Sampling

- a. **Personnel Requirements:** 2 personnel
- b. **Equipment Requirements:** 125 ml jars with lids, gloves, notebook, pens and pencils, hand-held GPS (2).
- e. **Form:** Location and sample number recorded in study notebook
- c. **Methodology:**

- i. Seven soil samples will be collected at each four intervals (28 samples total) according to the following schedule:

One complete set of samples ≤ 7 days prior to first application

One complete set of samples 14-17 days after first application

One complete set of samples 24-27 days after first application

One complete set of samples 54-57 days after first application

- ii. One person will be responsible for all soil sample collection. They will sample soil in 7 randomly selected locations of high crab density areas that were exposed to aerial broadcast. The locations will be documented using a GPS device. Individually labeled, chemically cleaned 125 ml jars will be filled by using the jar to scoop the soil. Once the jar is full, the top will be secured and the sample will be refrigerated. Samples will be shipped cold to USDA/APHIS/WS/NWRC field station in Hilo, Hawai'i and then on to the NWRC in Fort Collins for analysis.

E. Efficacy Monitoring:

- a. **Personnel Requirements:** 2 personnel daily
- b. **Equipment Requirements:** Hagurama cage traps (300), grated coconut, chunk coconut, radio transmitters, measurement tools, telemetry receivers, Yagi antennas, hand held GPS units, Ziplock bags, sharpies, notebook, pens and pencils, anesthesia equipment.

f. **Form:** All information recorded in study notebook

c. **Methodology:**

i. Monitoring Sites will include:

Camp area (15 radio-collared rats)
Aviation Island (10 radio-collared rats)
Strawn Island (5 radio-collared rats)

- ii. Radio-collared rats will be monitored before, during and after rodenticide baiting to measure the proportion that die or survive over the bait exposure period.
- iii. Prior to baiting, rats will be live-captured using Haguruma® cage traps. Between 25-100 traps will be set at each site; the exact number and layout (transects, grid pattern) will be determined upon arrival. We will prebait the trapping area with grated coconut 3 days prior to setting the traps baited with coconut chunks. Traps will be set as soon as possible after sunrise and checked within 24 hours to minimize the time captured animals spend in traps. A total of 30 rats of approximate equal sex ratio will be radio-collared. A miniature radio transmitter (1.8g) tuned between 164-168MHz will be attached to the neck of the rat (SOP FP/HI 001.00). The anesthetized animal will be placed back in a wire mesh cage trap and observed until it recovers from the anesthesia (isoflurane gas), then released at its capture location (trap station). Date, capture trap number, sex, body weight, reproductive status (male testes ascend/descend; female vagina imperforate/perforate, lactating, pregnant as determined by tactile feel of fetuses) will be recorded for each capture. Injured or otherwise unhealthy rodents will be euthanized in the field by anesthesia overdose or cervical dislocation. Non-target vertebrate captures will be recorded and released onsite.
- iv. Radio-tagged rats re-captured during the initial capture/mark/release period will be released at the site of capture. Portable telemetry receivers and hand-held Yagi antennas will be used to locate radio-tagged rats. Animals will be located every other day for approximately 4 days prior to and up to 21 days after bait application to determine bait efficacy (mortality). Attempts will be made to pinpoint the target transmitter signal and its location recorded (GPS) to facilitate subsequent days searches.
- v. Carcasses opportunistically found within the study plots will be collected and examined for evidence of radio collars, bait consumption (presence of bait material in the stomach and GI tract) and symptoms of anticoagulant poisoning (hemorrhaging). Attempts will be made to recover confirmed or suspected mortalities (stationary for 3 consecutive days) of radio-tagged rats from underground burrows, in tree nests or other locations. The exact locations (GPS) of visual sightings or recovered carcasses will be recorded. The proportion of radio-tagged rats that succumbed to the baiting and those that survived will be calculated.

PALMTRA ATOLL RAT ERADICATION						DAYS AFTER 1ST BROADCAST																												
MONITORING ACTIVITIES						Pte	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Post	TOTAL	
Application Rate Ground Hoops		96	96																		96	96											384	
Application Rate Lagoon Hoops		75	75																		75	75											300	
Bait Fete Ground Hoops ¹		40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	960	
Bait Fete Canopy Palm Crowns ²		15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	360	
Bait Fete Crab Burrows Burrows		10																															10	
Focused Carcass Search Dayz [N Beach, Runway]		6	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	56	
Atoll Carcass Search Samples							1														1												4	
Shoreland Count Runway Samples							1									1						1											4	
Shoreland Count Atoll Samples							1										1																4	
Non-Target Sampling - Cecidus Pooled Samples ³		2					2				3						2				3						2	3			5		22	
Non-Target Sampling - Ants Pooled Sample ³		2					2				3						2				3					2	3			5		22		
Non-Target Sampling - Codrionides Pooled Samples ³		2					2				3						2				3					2	3			5		22		
Non-Target Sampling - Fish Individuals		30																															60	
Non-Target Sampling - Fiddler Crabs ³		2					2				3						2				3						2	3			5		22	
Non-Target Sampling - Hermit Crabs ³		2																															12	
Environmental Sampling - Water - Outside:		3					3															3											12	
Environmental Sampling - Water - Inside Lagoon:		6					6															6											24	
Environmental Sampling - Water - Brackish Pel Pond:		1					1															1											4	
Environmental Sampling - Water - Runway Pond:		1					1															1											4	
Environmental Sampling - Water - Fresh Water Wall:		1					1																1										4	
Environmental Sampling - Soil		7																			7												7	28
Efficacy Monitoring - Radio Collars - Strawn Locations		5					5				5						5				5						5							

10. Experimental Design and Statistical Analyses

Descriptive statistics such as means, presence/absence of rodenticide residue, and demographics of collected animals will be reported. Additional statistics will be conducted to answer additional questions, as they become relevant for future management of the fauna of Palmyra Atoll.

11. Standard Operating Procedures (SOPs) and Analytical Methods

SOP/Method No.	Title
FP 031.00	Live trapping, transportation to research facility, handling, and processing of <i>Rattus</i> spp.
FP 034.00	Recovery and handling of animals found dead during routine field activities
CH 011.00	Selected avian tissue preparation for chemical residue analysis
FP/HI 001.00	Anesthetizing, marking, and radio-tagging mongoose (<i>Herpestes auropunctatus</i>)
FP/HI 002.00	Anesthetizing, marking, and radio-tagging rats (<i>Rattus</i> spp.)
HS 004.00	Personal protective equipment
HS 013.02	Shipment of biological substances, animal specimens, and environmental test samples

12. List of Records to be Maintained

- A. Protocol and Amendments
 - 1. QA 1875 original protocol (no amendments currently)
- B. Correspondence, telephone logs and related records
 - 1. Amanda Meyer, PhD., U.S. Fish and Wildlife Service (email correspondence, notes of phone calls)
 - 2. Stacie Hathaway, U.S. Geological Survey (email correspondence, notes of phone calls)
- C. Data records including:
 - a. All attached data forms
 - 1. Bait drift form
 - 2. Ground bait form
 - 3. Canopy bait form
 - 4. Bait caching /crab burrow form
 - b. GPS metadata and stored coordinates on devices

- c. Daily field notebook records of all personnel
 - d. Laptop and computer files of spreadsheets and documents
 - e. Sample collection log
- D. Final Report
- E. _____

13. Cost Estimate for Each Fiscal Year

FINANCIAL PLAN				
FISCAL YEAR 2011				
BETWEEN NWRC & ISLAND CONSERVATION				
Project Title: Palmyra Atoll Project				
Salary & Benefits				
Principle Investigator				9500.00
Biologist				27567.00
Biologist				22151.00
Biological Technician				20899.00
Subtotal				80117.00
Travel				
	People	Rate		
Per Diem on Island	3	150		450.00
Travel Honolulu (FLT, per diem, overnight)	3	950		2850.00
Travel Fort Collins to Hilo and per diem	1	1200		11200.00
Subtotal				14500.00
Laboratory Analysis				
		Samples	Cost per sample	
Animal Tissues		190	275	52250.00
Gecko method development		1	5000	5000.00
Ant method development		1	5000	5000.00
Cockroach method development		1	5000	5000.00
Water Samples		48	100	4800.00
Soil Samples		35	100	3500.00
Bait Analysis		10	100	1000.00
Subtotal				76550.00
Subtotal				0.00
Printing				
Subtotal				0.00
Other Services				
Sample Shipping and supplies				1000.00
Subtotal				1000.00
Supplies/Materials				
Subtotal				0.00
Equipment				
Subtotal				0.00
Grand Subtotal				172167.00
APHIS Administrative Overhead (16.15%)				27804.97
Grand Total				199971.97

Table 2. Itemized financial plan for proposed project.

14. Human Health and Safety

SOP HS 004.0 will be followed to ensure the personal protection of the health and safety of individuals through the use of personal protective equipment as needed.

15. Staff Qualifications

All study participants have documentation on file, which verifies their training and qualifications for the work they will perform in this study, including SOP training logs. All SOPs and study specific training logs will be completed and documented in study or personnel records prior to participation in that aspect of the study.

16. Archiving

All raw data, documentation, records, protocols, specimens, correspondence and other documents relating to interpretation and evaluation of data, and final reports generated as a result of this study will be retained in the archives of the National Wildlife Research Center at Fort Collins, Colorado.

17. Protocol Amendments

Any changes in this protocol will be documented on the Study Protocol Amendment Form, reviewed by appropriate personnel (e.g., IACUC, IBC, ACP, QA, etc.), and signed and dated by the Study Director, Project Leader, Assistant Director, and for regulated studies the Sponsor. Amendments will be distributed to all study participants as appropriate.

18. References

Alifano, A. and A. Wegmann. 2010. The ecotoxicology and palatability of two rodenticide bait products: field-based assessment at Palmyra Atoll Island Conservation, Santa Cruz, California.

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Marks, Jeffrey S., T. Lee Tibbitts, Robert E. Gill and Brian J. Mccaffery. 2002. Bristle-thighed Curlew (*Numenius tahitiensis*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: <http://bna.birds.cornell.edu/bna/species/705doi:10.2173/bna.705>

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Wegmann, A. S. 2008. Fanna Island rat eradication site visit: technical report - August 2008. Island Conservation, Santa Cruz, California.

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19. Appendices

Indicate none or check attached appendices:

- ☐ None
- ☒ Animal Use Appendix
- ☒ Analytical Chemistry Appendix
- ☐ Column E Explanation
- ☐ Material Transfer Agreement
- ☐ Microbiological/Biohazardous Materials Formulation and Use Appendix
- ☒ NEPA and ESA Appendix
- ☐ Test, Control and Reference Material/Device Use Appendix
- ☐ Other: (include appropriate title) _____

☐ Collaborating institution is responsible for live animal phase; IACUC protocol & approval attached

Technician: _____

Date: _____

Post Application Monitoring

Technician: _____

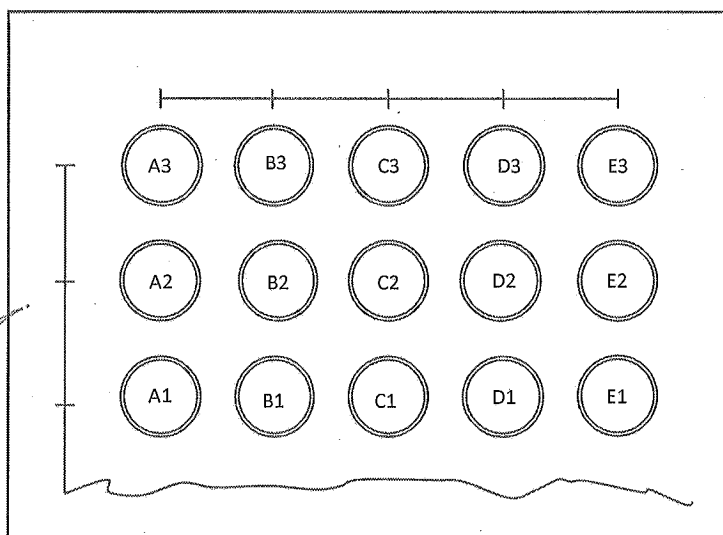
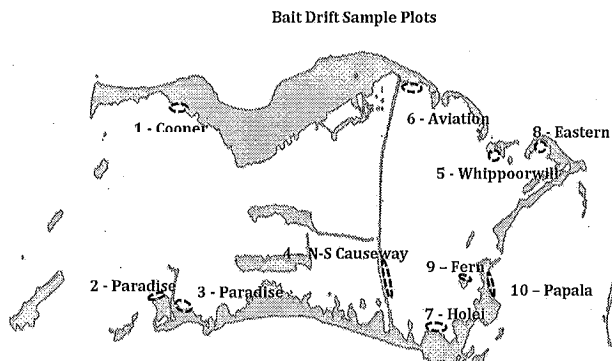
Date: _____

QA-1875

Bait Drift Form

Sampling Grid (way point)	Freezer ZipLock Storage Bag No.	Sample No.	Time Since Application (min.)	Pellet Weight (g)
		A1		
		A2		
		A3		
		B1		
		B2		
		B3		
		C1		
		C2		
		C3		
		D1		
		D2		
		D3		
		E1		
		E2		
		E3		

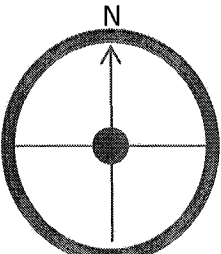
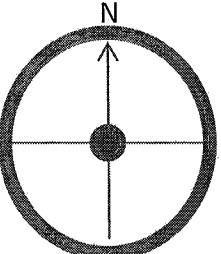
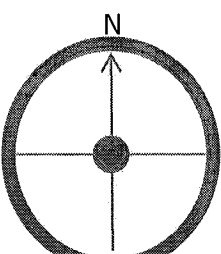
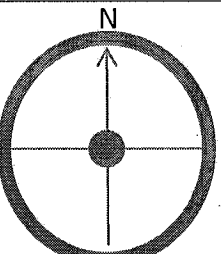
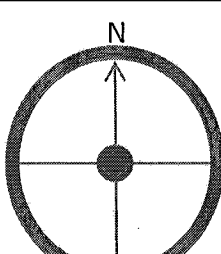
Notes



Palmyra Atoll Rat Eradication Project
Post Application Monitoring
QA-1875

Date: _____
Technician: _____

Ground Bait Fate Form

Sampling Area	Hoop Number	Pellet No.	Pellet Condition	Comment
		1		
		2		
		3		
		4		
		5		
		1		
		2		
		3		
		4		
		5		
		1		
		2		
		3		
		4		
		5		
		1		
		2		
		3		
		4		
		5		
		1		
		2		
		3		
		4		
		5		

LEGEND: Pellet Condition Scores

1	2	3	4	5	6	7
Bait hard, intact, whole	Bait hard, intact, partially gone	Bait soft, intact, whole	Bait soft, intact, partially gone	Bait mushy, disintegrated	Bait dry, disintegrated	Bait pellet gone

Palmyra Atoll Rat Eradication Project
Post Application Monitoring
QA-1875

Date: _____
Technician: _____

Canopy Bait Fate Form

Sampling Area	Tree Number	Pellet No.	Pellet Condition	Comment
	1	1		
		2		
		3		
		4		
		5		
	2	1		
		2		
		3		
		4		
		5		
	3	1		
		2		
		3		
		4		
		5		
	4	1		
		2		
		3		
		4		
		5		
	5	1		
		2		
		3		
		4		
		5		

LEGEND: Pellet Condition Scores

1	2	3	4	5	6	7
Bait hard, intact, whole	Bait hard, intact, partially gone	Bait soft, intact, whole	Bait soft, intact, partially gone	Bait mushy, disintegrated	Bait dry, disintegrated	Bait pellet gone

Date : _____
Technician: _____

Burrow Identification	Number of Pellets		Condition of Pellets	
	On Surface		On Surface	
	In Burrow		In Burrow	
	On Surface		On Surface	
	In Burrow		In Burrow	
	On Surface		On Surface	
	In Burrow		In Burrow	
	On Surface		On Surface	
	In Burrow		In Burrow	
	On Surface		On Surface	
	In Burrow		In Burrow	
	On Surface		On Surface	
	In Burrow		In Burrow	
	On Surface		On Surface	
	In Burrow		In Burrow	
	On Surface		On Surface	
	In Burrow		In Burrow	
	On Surface		On Surface	
	In Burrow		In Burrow	
	On Surface		On Surface	
	In Burrow		In Burrow	

Technician: _____

[illegible]

Animal Use Appendix

An "Animal" is defined as any vertebrate. "Use" includes manipulating the behavior of wild animals in their natural habitat, as well as capturing and/or handling animals.

Note: A consultation with the NWRC Attending Veterinarian must be performed prior to submitting this appendix to the IACUC for review. Allow a minimum of 2 weeks for the IACUC review process.

A. Animal Description

1) Animals:

Species, subspecies (if applicable):

Gecko species (*Gekkonidae*)

Black spot sergeant (*Abudefduf sordidus*)

Rats (*Rattus rattus*)

Breed, strain and substrain (if applicable): n/a

Total Number and Sex: see Table 1 in above protocol

Body weight range:

Gecko species: ~50-150 grams

Black spot sergeant: ~258 grams (152 mm = median adult length, body mass equation from Hawai'i Cooperative Fishery Research Unit)

Rats: 163 ± 4.5 grams (Wegmann 2009)

Age: unknown

B. Rationale for involving animals, for appropriateness of species, and for numbers

1) Rationale for involving animals:

Rats must be used to evaluate the efficacy of the operational activity of the aerial application of rodenticide. Non-target animals will be collected to assess the non-target effects of the action.

2) Rationale for appropriateness of the species to be used:

The species we list above are common on Palmyra Atoll, and therefore most likely to be affected by the action. The objective of monitoring these species is to evaluate the efficacy of the operational action and describe non-target effects at a species level.

3) Rational for numbers of animals to be used (include description of any animals to be obtained as extra if appropriate):

The sample size by species is presented in Table 1 in the above protocol. These sample sizes were determined as the minimum number needed to sample a given area while accounting for sample variability and attempting to collect a wide breadth of samples. Rat telemetry sample size should provide an indication of the speed of the eradication but will not take in to a rare event (resistance of a few individuals). Long term monitoring will be used to indicate success. Fish sample size were focused on the species most likely to consume bait and samples should detect residues. It is unclear what the residue level will be in the other species collected because they have not been sampled previously and methods are being developed for analysis.

C. Source

Animals will be trapped and collected from multiple sites on Palmyra Atoll. For details, see the methods section with figures documenting specific locations in the above protocol.

D. Method of identification of animals

Animals will be identified in concordance with existing relevant SOPs, such as rat handling, processing, and transportation (FP 031.00), and the completion of carcass forms (FP 034.00). All identification will be conducted by trained biologists and technicians.

E. Trapping/Collecting

A variety of trapping and collecting methods will be used in this study, dependent on the species and if the animal is live-trapped or collected dead. Details are presented in more detail in the methods section of the protocol above, however, we provide a list of trapping and collection by species here. Live trapping of geckos will be conducted using hand-capture and secondarily, commercially-available sticky/ glue traps. Live trapping of rats will be conducted using cage traps for radio collaring (SOP FP/HI 001.00, FP/HI 002.00, FP 031.00), of fish using spear guns and hoop nets. Carcass collection of rats will be opportunistic during routine surveys.

F. Transport

There is no planned transport of live animals in this study.

G. Handling/restraint

This study will use SOP FP/HI 002.00 will be used for the anesthetizing and handling of rats during radio collaring. Of note, is that a kill chamber containing an isoflurane-soaked cotton ball will be used for anesthetizing of the rats. Geckos will be anesthetized using an inhalant (isoflurane gas) and euthanized using decapitation followed by pithing. Live fish will be euthanized by stunning, followed by decapitation and pithing.

H. Quarantine

There is no expected need for quarantine of animals in this study.

I. Housing/maintenance

There is no housing of animals expected in this study.

J. Dietary contaminant exposure

There is no expected dietary contaminant exposure in this study, and it is non-applicable due to there being no housing in this study.

K. Disposition of animals

In line with the major objectives of this study, to determine the adverse impacts, short term negative impacts of the rodenticide application, as well as to determine the pathways of rodenticide exposure to non-target species, there is a strong component of species collection in this study. Therefore, non-target individuals which are ill or injured will be euthanized, collected, and used in the study. Target species (rats) which are ill or injured will also be sampled. As detailed in the methods of the above protocol, live animals will be trapped or captured using commercially-available traps or picked up by hand, and euthanized for residue analysis. Dead carcasses of rats will opportunistically be collected during routine monitoring activities. The fate of dead animals sampled will be shipment to testing facilities (SOP HS13.02) for tissue sampling procedures,

followed by proper disposal of carcasses in concordance with the testing facilities' established procedures.

L. Animal pain or distress

1) Consultation with Attending Veterinarian:

Consult with the Attending Veterinarian in advance to address any animal care and use issues. The Attending Veterinarian will determine if any portion of the study might cause more than momentary or slight pain or distress. Consultation should include discussion of alternative procedures, sedatives, analgesics, anesthetics, surgery and euthanasia.

Note: Consult separately, and with appropriate advance notice, the Animal Facilities Supervisory Personnel for space allocation in designated Animal Facilities.

Name of Attending Veterinarian: _____ Gordon Gathright, DVM _____

Date of Consultation: _____ May 12, 2011 _____

2) Is this study expected to cause more than momentary or slight pain or distress as determined by the Attending Veterinarian?

☐ No

☒ Yes If yes, continue with the following items.

a) Alternative procedures:

Geckos: The primary method of gecko capture is using hand capture and this wouldn't cause distress. However, if this is unsuccessful, we will use sticky traps and this may cause momentary distress. To minimize the distress, we will check traps frequently (within 24 hours of being set, and subsequently within 24 hours of the last check). The sticky traps would be put out as late as possible in the evening and check them first thing in the morning. Night time checks of traps are not feasible in all areas due to safety concerns of moving without trails in areas with potential hazards (falls, debris, and UXO).

Rats and fish: There is no pain or distress expected for the sampled fish or rat species due to the stunning and/or anesthization.

b) Sedatives, analgesics, or anesthetics or Column E Explanation:

If sedatives, analgesics, anesthetics will be withheld, attach the **Column E Explanation Appendix** and complete items #4—6.

c) Surgery:

M. Euthanasia

Euthanasia methods used include the stunning, decapitation, and pithing of black spot sergeant fish and anesthetization and decapitation followed by pithing of gecko species. Also, rats will be euthanized by either cervical dislocation or anesthetic overdose of isoflurane gas.

N. IACUC Approval

Date of IACUC Approval Letter: ____ 25 MAY 11 ____

O. Staff Qualifications

Experience with handling and maintenance of amphibians, birds, and small mammals, including euthanasia are documented on file in the form of resumes and CVs at the home institution of the following USDA personnel:

1. Kathleen Hayes, Technician
2. Thomas McAuliffe, Technician
3. William Pitt, Project Leader/Study Director
4. Are Berentsen, Wildlife Biologist

In addition, CVs are on record for non USDA Personnel.

1. Amanda Meyer, PhD. USFWS, Refuge Manager
- 2.
3. Stacie Hathaway, USGS Biologist

NEPA and ESA Appendix

A categorical exclusion (CE) is based on consideration of all environmental issues relevant to this study, including consideration of cumulative impacts on wild animals and other environmental parameters, such as removal caused by the study combined with other reasonably foreseeable removals by other causes (e.g., sport harvest, wildlife damage management actions, and any other known causes of mortality) pursuant to APHIS NEPA Implementing Procedures at 7 CFR Part 372.5(c)(2)(i). Examples of projects which would likely require more than a CE include, field trials that will have future effects (the registration of chems.), projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts.

This study qualifies for a Categorical Exclusion because:

☐ It is a research and development activity that will be carried out in laboratories, facilities, or other areas designed to eliminate the potential for harmful environmental effects--internal or external--and to provide for lawful waste disposal and does not include the use of free-ranging wildlife.

☒ It is a routine measures activity, such as surveys, sampling that does not cause physical alteration of the environment

☐ It includes the lawful use of chemicals, pesticides, or other potentially hazardous or harmful substances, materials, and target-specific devices or remedies, however such use will:

☐ A) be localized or contained in areas (<10 acres) where humans are not likely to be exposed, and is limited in terms of quantity

☐ B) not cause contaminants to enter water bodies

☐ C) not adversely affect any federally protected species or critical habitat

☐ D) not cause bioaccumulation

☐ This study does not qualify for a Categorical Exclusion.

Will this activity occur anyway even without involvement by NWRC?

☒ No

☐ Yes If yes, describe why this activity will occur and attach written confirmation from those conducting activity.

Address the potential to impact target species populations (including *cumulative impacts* of all activities on such populations, where relevant) and steps to be taken to minimize it.

In line with the major objective of documenting that the rat removal action has successfully removed all rats from Palmyra, there will be a large-scale impact on the target species (rats) population in the form of eradication. The overarching goal of the bait application is to completely eradicate rat species from Palmyra Atoll. To minimize cumulative impacts, such

as environmental impacts of the actions on target species, personnel will be collecting water and soil samples to access for rodenticide residue present in the environment. Impacts to target species as a product of their monitoring may include temporary distress during capture, anesthetizing, and radio collaring. To minimize negative impacts to the rats, we will adhere to the established SOP # FP/HI 002.00.

Address the potential to impact non-target species populations (including *cumulative impacts* on such populations, where relevant) or non-target domestic animals (e.g. pet cats, ducks, etc.) and steps to be taken to minimize it.

In line with the objectives of documenting adverse impacts, or lack thereof, to Palmyra's biota caused by the rat removal action, documenting short term negative impacts, or lack thereof, to Palmyra's biota caused by the rat removal action, and establishing clear pathways by which non-target species could be exposed to the rodenticide employed to remove rats from Palmyra, and measure rodenticide exposure levels for key non-target species, this study will be assessing non-target effects of the prior action of bait application via aerial broadcast. All expected non-target species will be collected live and euthanized, or collected as dead carcasses as they are found. They will be analyzed for chemical residue to determine the population level impacts of the action of aerial broadcast of rodenticide on Palmyra. There are no foreseen secondary non-target effects to the monitoring of the non-target effects of the prior action of bait application.

Effects on T&E species and eagles:

Could study result in the disturbance, harassment, capture or death of a state or a federally listed threatened or endangered species or the possible incidental take of eagles?

☒ No

☐ Yes If yes, describe species, potential impact and measures to be taken to minimize impact:

☐ Other: *Highly unlikely (risk is negligible) because*

Consultations:

Did you consult with a state or federal agency specifically on this action.

☒ No

☐ Yes If yes, describe the date/mode/contact person and outcome of this consultation:

Landowner Permission: Do you have an agreement or permission to conduct the action on property owned or managed by a land manager or landowner.

- ☐ No, permission not needed because:
- ☒ Yes
- ☐ Other: *Permission will be obtained prior to entering property.....*

Analytical Chemistry Appendix

If chemical analysis by NWRC Analytical Chemistry is required, a consultation with the NWRC Analytical Chemistry Project (ACP) Leader is needed. List the approximate number of samples to be analyzed, the storage conditions, the Analytical method and the name and date of the ACP consultation.

A. Number of samples to be analyzed (by type): see Table 1 below

B. Storage conditions (temperature, container type, light/dark, duration):

All samples will be shipped frozen in concordance with SOP HS513.02 and they will remain frozen at the testing facility at the NWRC facility in Fort Collins, CO until processed.

C. Method title and number:

New methods were developed for cockroaches, ants, gecko, water, and soil samples and the methods number is pending. This study will use the established method of chemical residue testing for crab species, # 139-A.

D. ACP Leader approval: _____ Dave Goldade _____ **Date:** __27 April 2011__
(attach email or letter of concurrence from Analytical Services Project Team Leader)

If chemical analysis will be made by a laboratory outside of NWRC, include A-C above and attach the method to be used.

