Study Title: Monitoring diphacinone residues after an eradication of Polynesian rats from Lehua Island, Hawaii

NWRC Study Director: Shane R. Siers

Approved NWRC Project: Methods and strategies to manage invasive species impacts to agriculture, natural resources, and human health and safety

	SIGNATURE	DATE
NWRC Study Director:	SHANE SIERS 2017.07.24 14:40:30 -10'00'	24 JUL 17

Study Director's position (check one):

- ☒ Project Leader
- ☐ Research Scientist (non-project leader)
- ☐ Biologist/Chemist/Technician
- ☐ Student: NWRC Representative/Contact: _____
- ☐ Visiting Scientist: NWRC Representative/Contact: _____

	SIGNATURE	DATE
Concur: **NWRC Research Project Leader:**	SHANE SIERS 2017.07.24 14:41:10 -10'00'	24 JUL 17
QAU Review and Processing: **NWRC Quality Assurance:**		7/24/17
Concur: Acting **NWRC Assistant Director:**		7/25/17
Approved: **NWRC Director:**		7/25/17

REGULATORY CONSIDERATIONS

Analytical Chemistry
Will chemical analysis be required of the NWRC Chemistry Lab Unit?
☐ No ☒ Yes – **Attach the Analytical Chemistry Appendix.**

Will the services of the NWRC Formulation Scientist be needed?
☐ No ☐ Yes – **Attach the Formulation Support Appendix.**

Animal Use
Will the study include the use of animals?
☐ No ☒ Yes – check all that apply below.
- ☐ Live animals will be used at an NWRC facility. **Attach the Animal Use Appendix.**
- ☒ Handling animals or manipulating the behavior of wildlife in the field. **Attach the Animal Use Appendix.**
- ☐ Collaborating institution is responsible for all or part of live animal phase. **Attach the collaborating institution's protocol and IACUC approval.**
- ☐ Study will be conducted using privately owned animals. **Attach "Consent for the Use of Privately Owned Animals" form (SOP AD025).**
- ☐ No manipulation of the behavior of wildlife in the field (observation only). **No appendix needed.**
- ☐ Samples or data opportunistically collected from ongoing operational activities. **No appendix needed.**

Biological Laboratories (BioLabs) Support
Do you anticipate you will require space, equipment, or personnel from the NWRC Biological Laboratories Unit?
☒ No ☐ Yes – **Date of consult with Laboratory Specialist:** Click here to enter text

Microbiological/Biohazardous Materials
Will any Microbiological/Biohazardous Materials be used?
☒ No ☐ Yes – **Attach the Microbiological/Biohazardous Materials Use Appendix.**

Intellectual Property (IP) Considerations
Do any of these situations apply to this study?
- The condition of confidentiality between you and your collaborator would facilitate open discussions and collaboration.
- This research involves the exchange or transfer of material(s) between the NWRC and your collaborators.
- This research includes existing IP and/or could lead to the development of new IP.

☒ No ☐ Yes – Consult the NWRC Technology Transfer Coordinator. **Date of consult:** Click here to enter text

Federal Environmental Statute Considerations
Will this activity involve a field component and meets any of the following conditions?
The field component will occur on Federal land, is funded with Federal money, and/or involves Federal personnel.
☐ No ☒ Yes
- Complete and **Attach the Endangered Species Act Appendix (ESA)** and
- Complete and attach the **National Environmental Policy Act Appendix (NEPA).**

Regulated Product Registration Considerations
Does this activity involve the transfer OR testing of any pesticide, vaccine, drug, diagnostic kit, or pest control or medical device, or their components, including products still in the research and development stage?
☒ No ☐ Yes - Consult with the NWRC Registration Manager regarding any regulatory requirements.

As determined during this consultation, check the applicable regulatory standards.
☐ none ☐ EPA GLP ☐ FDA CVM GLP ☐ USDA CVB GLP-like ☐ OECD GLP
☐ other: Click here to enter text

DESCRIPTION OF ACTIVITIES

NWRC Collaborators:

Name	NWRC Project	Contribution to study
Shane R. Siers	Island Invasives	Study design; project coordination; data analysis; report writing
Dean K. Foster	Island Invasives	Protocol preparation; logistics; field data collection; report writing
Aaron B. Shiels	Rodent Project	Consultation; report review
[TBD]	Chemistry Lab Unit	Analytical chemistry; report writing

Non-NWRC Collaborators:

Name	Affiliation	Contribution to study
Patricia Baiao	Island Conservation	Project coordination; report review
Gregg Howald	Island Conservation	Consultation; report review
"Brand" Reese Phillips	USFWS	FWS prepared the project environmental assessment, NWRC will be a signatory.

Study location(s):

Name	Address	Activities at this location
Lehua Island	County of Kauai, Hawaii	Sample collection
NWRC Chemistry Lab Unit	4101 LaPorte Ave, Fort Collins, CO	Diphacinone residue analysis

Funding Source:

Source of Funds	APHIS Program	Name of Non-APHIS Collaborator	$ Amount
Internal (NWRC)	Hawaii Field Station		$47,000
Non-APHIS Collaborators			

Study Schedule:

Proposed study start date: 30 July 2017

Proposed study end date: 31 December 2017

Proposed archive date: 30 June 2018

Background/Justification:

On all but the smallest of islands, successful rodent eradication efforts employ the landscape-scale application of toxic baits. The rationale for such short-term contaminant inputs is that the environmental and human health risks of toxicant use are offset by the long-term ecological and societal benefits of invasive rodent removal. The maintenance of this rationale requires that we continue to test assumptions about the actual primary and secondary adverse impacts of rodenticide use.

The state of Hawaii is undertaking a rat eradication action on Lehua Island, Hawaii, in cooperation with USFWS and Island Conservation and USDA NWRC. The Fish and Wildlife Service will be the lead agency for writing the environmental compliance documents (EA) and a final EA and FONSI have already been completed. Island Conservation will be the lead entity performing the actual application and monitoring of rodenticide baits. NWRC will be the lead agency for collecting environmental samples to evaluate diphacinone inputs into the environment and fauna. The proposed implementation plan for the August 2017 eradication of Polynesian rats (*Rattus exulans*) from Lehua Island calls for three applications of a rodenticide bait containing 0.005% diphacinone, an anticoagulant, in a cereal-based bait matrix.

Previous sampling: A similar monitoring effort was implemented by the US Geological Survey in conjunction with an attempted eradication in January of 2009 (Orazio et al. 2009). During this previous testing, no diphacinone residues were detected in any seawater, soil, invertebrate or fish samples. For the sake of comparability of results, this sampling scheme would be replicated for the proposed eradication attempt with minor modifications to increase the focus on species routinely collected for human consumption.

Research Objective/Hypothesis:
The purpose of this environmental monitoring plan is to assess the potential persistence of diphacinone in various environmental compartments subsequent to this eradication action.

Methods, Procedures and Experimental Design:
Analytical chemistry: Diphacinone residues would be assayed and quantified by liquid chromatography and mass spectrometry (LC-MS/MS) at the USDA APHIS WS NWRC Chemistry Lab Unit in Fort Collins, CO. Detection and quantitation limits for each sample type would be established during analysis and compared to the limits established during the previous assays that employed high performance liquid chromatography (HPLC) followed by ultraviolet-visible photodiode array absorbance (PDA) detection.

Sampling sites: During the 2009 sampling effort samples were collected at the three sites depicted in Figure 1. These locations were the only places where the shore-based sampling crews could safely access the shoreline and where there would be potential public use of Lehua's near-shore resources. These sites also represent the most conservative circumstance for detection of residues as the largest drainage gulches occur on the south slope of the island and enter the sea in this area of greatest human use. We also anticipate collecting samples from these sites. A more detailed view of these locations can be seen in Figure 2. During the proposed eradication attempt, we would consider alternative, dispersed sampling sites, depending on ocean and weather conditions and risk to human safety.

Barring the use of alternate collection sites the water, limpet, crab, and fish samples will be collected within 25-50 meters from the following coordinates, whereas the soil samples will be collected 20-30m upslope from the same sites along gulch bases excluding the gulch containing structures:

Site 1: 22.015005°, -160.096735°
Site 2: 22.014903°, -160.095862°
Site 3: 22.015097°, -160.095336°
Locations are derived from GoogleEarth© WGS 84, decimal degrees.

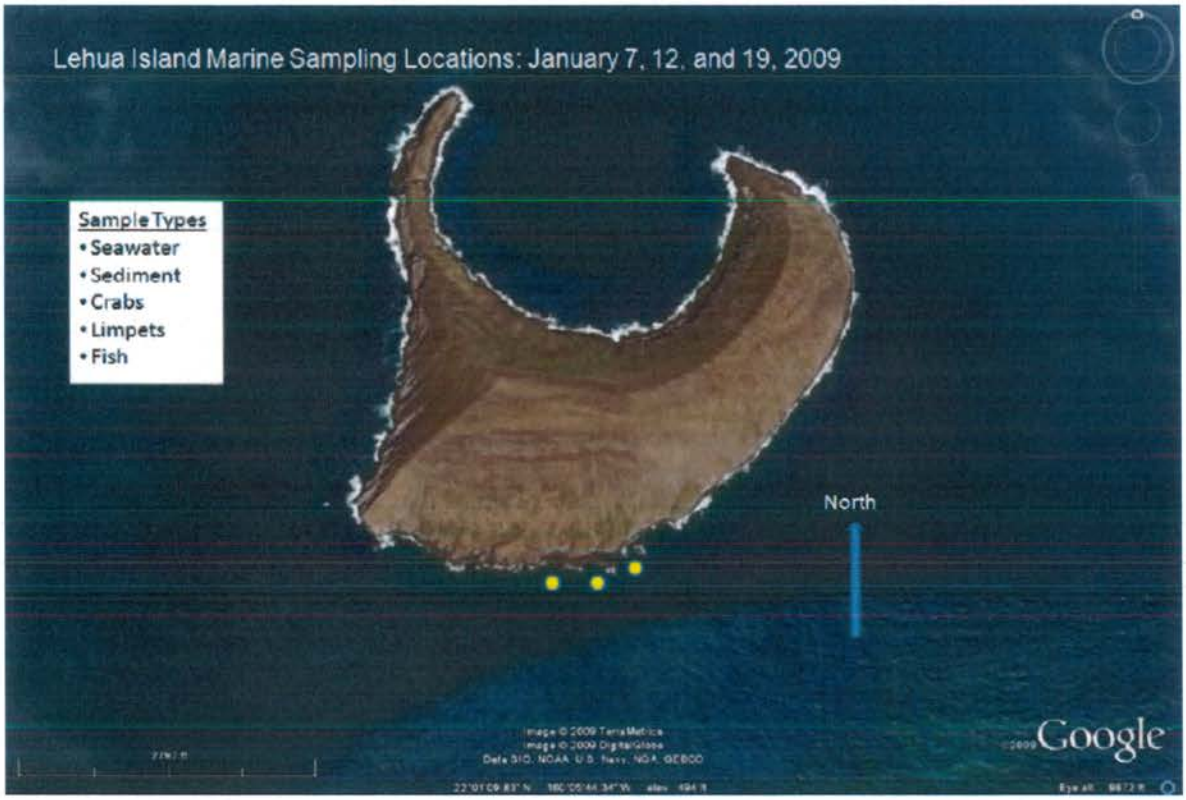

Figure 1. (From Orazio et al. 2009) Lehua Island, Niihau, Hawaii (Created from GoogleEarth image) showing the three sampling sites (yellow dots) on the south of the island. Indicated sample types were collected at each sampling location.

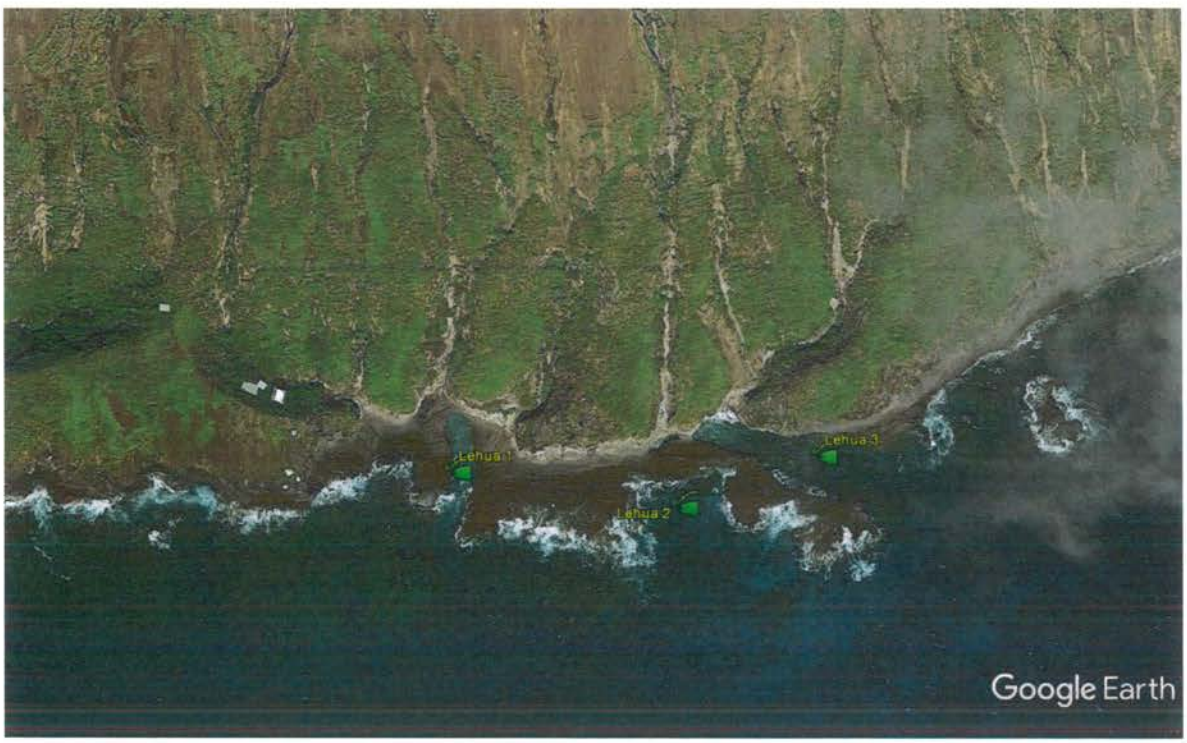

Figure 2. Lehua Island, Niihau, Hawaii (Created from GoogleEarth image) showing a zoomed in view of the three sampling sites (fishing icons) and nearby drainage gullies and structures.

Sampling occasions: Specimens will be collected from each of the three selected sites prior to bait application (to establish a baseline), then again one to four days following each of the three planned applications, followed by a final sampling one to two weeks later, for a total of five sampling occasions.

Sample handling: Animal samples will be stored whole and necropsies will not occur on Lehua Island to minimize sample contamination concerns as there will not be a clean laboratory on site. All soil, water, and carcass samples will be refrigerated following collection using iced coolers due to the island's remoteness. Approximately weekly the samples will be shipped cold or preferably frozen to NWRC headquarters in Fort Collins Colorado for chemical analysis. Chain of custody forms (plus seals if necessary) will be used to maintain sample integrity en route to the laboratory. Fish will be euthanized by manually applied blunt force trauma followed by pithing as described in AVMA 2013. Since we are collecting the fish for diphacinone residue analysis during an active eradication effort it is imperative that we not contaminate the carcasses during the euthanasia action (i.e. dirty knife or pithing rod). Limpets and crabs will be euthanized by either placing them in labeled plastic bags and then chilling and freezing or as a second choice anesthetized first by immersion in eugenol solution followed by euthanasia via chilling or preferably freezing. Immersing aquatic invertebrates in ethanol prior to chilling is inappropriate for our study at it could possibly could draw-out the analyte and invalidate the chemical analyses by falsely reporting negative or diminished residue concentrations.

The AVMA 2013 Guidelines state: "...Because of the variety of situations that may be encountered, it is difficult to strictly classify methods for termination of free-ranging wildlife as acceptable, acceptable with conditions, or unacceptable. Furthermore, classification of a given method as a means of euthanasia or humane killing may vary by circumstances. These acknowledgments are not intended to condone a lower standard for the humane termination of wildlife. The best methods possible under the circumstances must be applied, and new technology and methods demonstrated to be superior to previously used methods must be embraced" Thus, we acknowledge that we may need to be adaptable to changing field conditions and the wildlife we encounter and vary the humane euthanasia methods prescribed above due to unforeseen circumstances or to protect human health and safety.

Seawater and soil sampling: Water samples will be collected in 1 liter bottles with Teflon-lined lids whereas soil samples will be collected in 125 ml bottles of same construction. Bottles will be filled using only the bottles to collect material, i.e. do not use lids, cups, or trowels to fill bottles.

Water Sampling

a. Personnel Requirements: 1 personnel
b. Equipment Requirements: gloves, 1-liter water bottles, sharpies, foam coolers notebook, pens and pencils
c. Form: Location and sample number recorded in study notebook
d. Methodology:

i. One-liter water samples will be collected at the following locations:

One sample from each of three sites shown on Figure 2.

ii. Sampling will be conducted at five intervals, for a total of 15 samples:

One complete set of samples prior to the first application
One complete set of samples 1-4 days after the first application
One complete set of samples 1-4 days after the second application
One complete set of samples 1-4 days after the third application
One complete set of samples approximately 2 weeks following the final application.

iii. One person will be responsible for all water sample collection but may be assisted by properly trained personnel. The collector will wear protective gloves during all water sampling. Chemically cleaned 1-liter Nalgene © 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 almost completely leaving headspace for freezing expansion and the top will be secured. Samples will be chilled after collection. Water samples will be packed in commercially available coolers kept chilled during shipment to USDA/APHIS/WS/NWRC field station in Hilo Hawaii, frozen for short term storage, and then shipped frozen to the NWRC in Fort Collins for analysis.

Soil Sampling

a. Personnel Requirements: 1 personnel
b. Equipment Requirements: 125 ml bottles with lids, gloves, notebook, pens and pencils, hand-held GPS.
c. Form: Location and sample number recorded in study notebook
d. Methodology:

i. Soil samples will be collected 20-30 m from the water's edge adjacent to the three water sampling sites.

ii. Three soil samples will be collected at each of 5 intervals (15 samples total) according to the water sampling schedule:

ii. One person will be responsible for all soil sample collection but may be assisted by properly trained personnel. They will sample soils in 3 locations documented using a GPS device. Individually labeled, chemically cleaned 125 ml glass or plastic bottles will be filled by using the bottle to scoop the soil. A composite soil sample will be collected by scooping down 5-12 cm within the base of gulches that drain into the sea. Once the bottle is full, the top will be secured and the sample will be stored cool. Samples will be packed in commercially available coolers kept chilled during shipment to USDA/APHIS/WS/NWRC field station in Hilo Hawaii, frozen for short term storage, and then shipped frozen to the NWRC in Fort Collins for analysis

Limpet ('Opihi') sampling: Limpets are single-shelled marine gastropod mollusks (*Cellana* spp.) and a valued human delicacy. They graze on algae growing on rocky substrates in intertidal zones. Composite samples of 5-8 whole limpets would be collected at each of the sites during each sampling period. The intertidal habitat of limpets is inherently risky for human collection activities; successful collection during the targeted sampling interval is dependent upon tide and wave conditions and would not be conducted when human safety is at risk.

Limpet Sampling

a. Personnel Requirements: 2 personnel
b. Equipment Requirements: clean gloves or clean hands, plastic bags, sharpies, coolers, notebook, pens and pencils, and appropriate implement for prying shells from substrate
c. Form: Location and sample number recorded in study notebook
d. Methodology:

i. Composite limpet samples (5-8 individuals) will be collected at the following locations:

One composite sample from each of three sites shown on Figure 2. If limpets are unavailable or unsafe to collect at these sites alternates will be chosen and the new coordinates entered into the study notebook.

ii. Three composite limpet samples will be collected at each of 5 intervals (15 samples total) according to the water sampling schedule:

iii. One person will be responsible for all limpet collection but should be aided by at least one properly trained assistant. Limpets will be collected by hand preferably at low tide and 5-8 limpets will be humanely euthanized and placed into a labeled plastic bag. A best management practice for safe limpet collection is to have a spotter to warn the collector of the approach of a set of large waves, leave the intertidal area during large waves, and then continue collection between sets. We will be collecting limpets in the protected channel between Lehua and Niihau Islands which should be the safest area of the island for intertidal work. Samples will be packed in commercially available coolers kept chilled during shipment to USDA/APHIS/WS/NWRC field station in Hilo Hawaii, frozen for short term storage, and then shipped frozen to the NWRC in Fort Collins for analysis.

Natal lightfoot crab ("A'ama') sampling: 'A'ama (*Grapsus tenuicrustatus*) is a food source commonly eaten raw at parties. These crabs also occur in dangerous rocky intertidal zones, and are difficult to catch. Composite samples of 2-3 crabs would be collected when risk of human injury is low. These crabs can be pinned with long poles or blinded with flash lights at night and collected by hand.

A'ama Crab Sampling

a. Personnel Requirements: 2 personnel
b. Equipment Requirements: clean gloves or clean hands, plastic bags, sharpies, coolers, notebook, pens and pencils, devices to pin or entrap crabs above the water line if desired
c. Form: Location and sample number recorded in study notebook
d. Methodology:

i. Composite crab samples (2-3 individuals) will be collected at the following locations:

One composite sample from each of the intertidal zones of the three sites shown on Figure 2. If a'ama crabs are unavailable or unsafe to collect at these sites alternates will be chosen and the new coordinates entered into the study notebook.

ii. Three composite crab samples will be collected at each of 5 intervals (15 samples total) according to the water sampling schedule:

iii. One person will be responsible for all crab collection but could be aided by one properly trained assistant. Crabs will be collected by hand and 2-3 crabs will be humanely euthanized and placed into a labeled plastic bag. We will be collecting crabs in the protected channel between Lehua and Niihau Islands which should be the safest area of the island for intertidal work. Composite crab samples will be packed in commercially available coolers and kept cold or preferably frozen during storage and shipment to USDA/APHIS/WS/NWRC field station in Hilo, Hawai'i and then on to the NWRC in Fort Collins for analysis.

Fish sampling: The potential for toxic residues in reef and game fish is the greatest public concern associated with rodenticide use on Lehua. For this reason, we will place greater emphasis on fish sampling than in the 2009 effort. We will attempt to obtain multiple samples from the following classes of fish: 1) resident (non-pelagic) reef fish that were observed interacting with placebo bait pellets during prior site visits; these are likely to constitute a 'worst case scenario' for fish likely to directly consume higher levels of rodenticide (Table 1); 2) black triggerfish (*Melichthys niger*); a die-off of this species on the coast of Niihau coincident to the 2009 eradication attempt has been attributed to toxic algae, unrelated to rodenticide use, though public impression that it was caused by diphacinone poisoning persists despite a total lack of supporting evidence; 3) prized near-shore game fish, particularly higher trophic level predators more likely to bioaccumulate toxins; effort to collect predatory fish may be delayed until the later sampling periods to allow time for bioaccumulation (if any occurs). While this would be our objective, it must be recognized that reliable and species-specific collection of fish is likely to be very difficult, and the actual samples collected would likely be highly variable. Fish will be collected by cast net, hook and line, and spear, with sampling efforts restricted to periods of safe ocean conditions. Spear gun tridents or similar will be cleaned between fish captures to reduce the risk of fish tissue cross-contamination. Toxic residues in vertebrates are most highly concentrated in liver tissue; however, liver comprises a small component of fish mass, and "extrapolation from liver to muscle contaminant levels is fraught with uncertainty, and [such] data are essentially useless for this purpose" (Ahmed 1991). In most cases, livers of game fish are discarded. For the sake of balancing the most extreme potential concentrations with the realistic potential for exposure, fish large enough to be filleted will have both liver and muscle tissue sampled and tested independently; smaller fish, more likely to be included whole in soups and stews would be homogenized in the lab for a whole-body estimate of residue concentrations.

Fish Sampling

a. Personnel Requirements: 2 personnel
b. Equipment Requirements: clean gloves or clean hands, fishing poles, spear gun(s) plastic bags, sharpies, coolers, notebook, pens and pencils.
c. Form: Location and sample number recorded in study notebook
d. Methodology:

i. Whole fish will be collected at the following locations:

Fish will be collected from each of three sites shown on Figure 2. If fish of the correct type are unavailable or unsafe to collect at these sites alternates will be chosen and the new coordinates entered into the study notebook.

ii. Sampling will be conducted at five intervals for both "pellet consumers" and black trigger fish, for a total of 30 fish samples (15 fish per type). Five near-shore game fish will also be collected at each site approximately 2 weeks after the last bait drop for a total of 15 more fish. Trigger fish and game fish carcasses will be split into muscle and liver tissue samples by the analytical laboratory raising the total fish samples analyzed to 75.

Three "pellet consumers" and 3 black trigger fish prior to the first application
Three "pellet consumers" and 3 black trigger fish 1-4 days after the first application
Three "pellet consumers" and 3 black trigger fish 1-4 days after the second application
Three "pellet consumers" and 3 black trigger fish 1-4 days after the third application
Three "pellet consumers", 3 black trigger fish, and 15 near-shore game fish ~2 weeks following the final application.

iii. One person will be responsible for all fish collection but could be aided by one or more properly trained assistants. Fish will be collected by hook and line or spear gun (cleaned between fish) if necessary. Locally collected baits should be avoided to prevent possible diphacinone inputs into fish samples. All fish will be placed whole into an individually labeled plastic bag. Necropsies or tissue collections will not take place on island to avoid diphacinone contamination. We will be fishing in the protected channel between Lehua and Niihau Islands which should be the safest near shore area of Lehua Island for spear fishing activities. Sampling will not occur under unsafe conditions. Fish samples will be packed in commercially available coolers and kept cold or preferably frozen during storage and shipment to USDA/APHIS/WS/NWRC field station in Hilo, Hawai'i and then on to the NWRC in Fort Collins for analysis.

Table 1. During a 2015 inert bait trails on Lehua, bait was hand broadcast into coastal areas containing rock shelves at depths from one to three meters and into open sandy bottomed areas with depths up to six meters. Observers conducted time-constrained surveys of fish species that came into contact with, mouthed or consumed the bait. Following are the common, Hawaiian, scientific names and observations of species interacting with inert pellet bait and considered to be fish caught and eaten locally:

Common Name	Hawaiian Name	Scientific Name	Observation
Achilles Tang	Pākuiku`i	*Acanthurus achilles*	Contact
Orange-band Surgeonfish	Na`ena`e	*Acanthurus olivaceous*	Contact
Bluestripe Snapper	Taape	*Lutjanus fulvus*	Consumed bait*
Hawaiian Hog Fish	A`wa	*Bodianus albotaneniatus*	Consumed bait*

** It was determined during the initial survey that species mouthing pellets could not easily be distinguished from those that consumed pellets and these two categories were then combined into the single category "consumed." Therefore, whether these fish would actually consume bait is not established.*

Non-target carcass surveys: Throughout the course of field activities associated with eradication efforts, any non-target organisms (species other than rats) found dead will be collected and submitted for chemical residue analysis to assess whether the organism had been exposed to rodenticide intoxication (with birds being the primary taxa of concern). Passive surveillance will occur through alertness of all personnel at all times, collecting and submitting all carcasses found while conducting all other eradication monitoring activities. Active surveillance will occur through the assignment of carcass search transects throughout terrestrial habitat and along coastlines adjacent to the encampment area as ocean conditions permit. Carcass search personnel will be equipped with binoculars to extend their field of view. The level of survey effort will be balanced with other demands on field personnel time. Significant effort will be applied to pre-drop carcass searches to document natural mortality and to remove carcasses that could later be confused with mortalities due to rodenticide treatment. To the greatest extent possible, non-target carcasses will be necropsied by veterinary professionals to evaluate evidence as to whether the mortality was associated with rodenticide ingestion or not.

Chain of custody: Sample identification, date, location, and collector data will be recorded and maintained with the samples, along with a documented chain of custody between the source location and the NWRC Chemistry Lab Unit in

Fort Collins. Tissue samples will not be collected on-island, to minimize the risk of contamination of samples through contact with equipment, dust, or hands contaminated with toxin residues.

Sample analysis prioritization: Chemical residue analysis is costly. Current availability of funds is limited, and additional future funding is uncertain. We will strive for a comprehensive sampling of specimens, and have abundant samples on hand for future testing as funding becomes available. Priority will go to analysis of samples more directly related to common human consumption practices (e.g., Opihi and popular game fish) and listed non-target species. Specimens from the sample collected after the last application are likely to have the highest accumulated contaminant levels (if any), with the most valuable information for inference on the highest risk of contamination in game fish, and will be prioritized for prompt analysis. If any residues are observed in that sample, the final sample (one or two-weeks post-application) will be of the next highest priority, to determine if residues still persist. It could be argued that the first and second post-application samples are of the least practical value for assessing overall project impacts and human health risks because they do not incorporate the full extent of toxin application (as the sample after the third application does) and does not inform the overall duration of residue persistence; chemical analysis of these samples could be foregone if adequate funding is not secured. It is likely that lab results will take eight weeks or longer to become available, with limited opportunity for expedited results.

Contingency sampling: Additional sampling materials and protocols will be on hand to respond to any unanticipated non-target mortalities beyond what might be expected within the action area (for example, the 2009 triggerfish die-off on Ni`ihau) to distinguish between diphacinone contamination and coincidental mortality events.

Sampling scenario example: Following is a summation of a potential sampling scheme. The total cost is based on the full-price estimate of $150 per sample. The cost of processing large groups of samples can be reduced by providing technician labor to assist in sample preparation.

SAMPLE	Pre-app	App 1	App 2	App 3	Post-app
Seawater	3	3	3	3	3
Soil	3	3	3	3	3
Crab	3	3	3	3	3
Limpet	3	3	3	3	3
Fish (pellet consumers)	3	3	3	3	3
Fish (triggerfish)*	6	6	6	6	6
Fish (larger predator)*	0	0	0	0	30
Non-target carcasses	6	6	6	6	6
TOTAL SAMPLES	165				
MAX LAB COST (@$150 ea.)	$24,750				

* Larger fish species will have two samples taken per fish (liver and muscle); these number indicate two sample per fish.

Rodenticide compounds: This monitoring plan is drafted under the assumption that rodent eradication would be attempted with diphacinone bait. If brodifacoum is used, this sampling strategy would be revised to account for the known increased persistence and bioaccumulation of chemical residues. Additional post-operational sampling may be required if residues persist after the last sampling period, and additional environmental compartments (e.g., insects and lizards) may be tested.

Statistical Analysis:

Data will be tabulated and reported with simple summary statistics and binomial confidence intervals for estimated proportions of samples contaminated.

Human Health and Safety Risk/Hazard Assessment:

SOP HS 004.00 will be followed as much as is possible to ensure the health of NWRC staff; however, collecting organisms within rocky intertidal zones on Pacific Islands exposes workers to unique health and safety risks not covered

in this SOP. Shoreline rocks can be both slippery and jagged and thus reef boots or similar grippy and tough foot wear is recommended. Knives or screwdrivers used to pry limpets from rocks should be blunt edged like butter knives to reduce the risk of a hand injury. It is recommended to work in pairs when collecting limpets with one person acting as a spotter to warn the collector of the approach of a set of large waves, the collector leaves the intertidal area during large waves, and then continues limpet collection between sets. Certain fish are quite spiny and it is best to handle them when alive with gloved hands. None of the targeted fish species are poisonous. Aama crabs lack large pinching claws and are somewhat delicate so using gloves to capture them isn't necessary to prevent injury. Birds found dead will be handled with gloved hands and double bagged and tagged according to SOP FP 034.00.
Biologist Dean Foster is a trained Masters level fishery biologist with extensive experience collecting fish in near shore environments including with the use of spears. If hook and line sampling proves ineffective then free-diving spearfishing will be employed and Dean will train any assistants.

Standard Operating Procedures (SOPs)/Analytical Chemistry Methods:

SOP/Method No.	Title
Method 163A	Determination of Diphacinone in Ramik Green® Pellet Baits and Chlorophacinone in Rozol® Grain Baits
HS 004.00	Personal protective equipment
FP 034.00	Recovery and handling of animals found dead during routine field activities
Click here to enter text	Click here to enter text

Cost Estimate for Each Fiscal Year*:

	FY-17	FY-18	FY-19	FY-20
A. Salary and Benefits	$11,500	$6,750		
B. Facilities (in addition to existing facility or space costs)				
C. Equipment				
D. Supplies	$1,000			
E. Animal Care Costs				
F. Operating Costs (travel, misc. services, etc.)	$3,000			
G. Chemistry Services (depends on actual number of samples)	$12,375	$12,375		
TOTAL	$27,875	$19,125		

***Distribution between in-kind NWRC contribution and external funding TBD**

Archiving:
The protocol, amendments, raw data, documentation, records, 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.

Protocol Amendments:
Any changes in this protocol will be documented prior to the change using the Protocol Amendment form, reviewed by the appropriate personnel, signed and dated. Approved amendments will be distributed to all study participants as appropriate.

References:
Ahmed, FE (ed.). 1991. Seafood Safety. National Academy Press, Washington, DC.

Orazio, CS, MJ Tanner, C Swenson, J Herod, P Dunlevy and RW Gale. 2009. Results of laboratory testing for diphacinone in seawater, fish, invertebrates, and soil following aerial application of rodenticide on Lehua Island, Kauai County, Hawaii, January 2009. US Geological Survey Open File Report 2009–1142, 15 pp. + appendix.

Other Pertinent Attachments: (list in order of appearance)

- Analytical Chemistry Appendix
- Animal Use Appendix
- ESA Appendix
- NEPA Appendix

ANALYTICAL CHEMISTRY APPENDIX

If chemical analysis by NWRC Analytical Chemistry is required, a consultation with the NWRC Chemistry Lab Unit (CLU) 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 CLU consultation.

A. Number of samples to be analyzed (by type):

Approximate counts (max):
Seawater: 15; soil: 15; crab: 15; limpet: 15; fish (whole body, liver, or muscle): 75; bird: 30

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

Soil, seawater, crab, limpet, fish, and bird: whole body or muscle and liver tissues collected under conditions free from potential rodenticide contamination, placed in plastic (Ziploc) bags, and stored chilled in coolers for up to 5 days before transfer to freezer. Stored frozen until shipped to HQ on dry ice or suitable substitute.

C. Method title and number:

Method 163A

D. Chemistry Lab Unit Leader consultation: ____David Goldade_____ Date: ___June 28th 2017____

☐ Chemical analysis will be performed by a laboratory outside of NWRC.
Include items A-C above and attach the method to be used as an appendix to this protocol.

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. Related Protocols:

List by number

QA 1875	Palmyra Atoll rainforest restoration project: rat eradication monitoring plan for alternatives B and C (aerial broadcast of 25W)
QA 2441	Wake Atoll fish tissue sampling and analysis three years after an island wide rodenticide application
QA 2523	Assessment of a hand-broadcast rodenticide bait trial to control rats in the Waianae Mountains, Oahu
QA 2546	Comparing efficacy and acceptance of two novel diphacinone formulations for control and eradication of Polynesian rats (*Rattus exulans*) and mice (*Mus musculus*) in conservation areas

B. Assurance of Non-duplication of studies

Provide an assurance that activities in this study do not unnecessarily duplicate previous experiments. If there is duplication, provide scientific justification why this study is necessary. List the databases searched, the date of the search, the period covered by the search, and the key words used or provide other procedures used in your determination.

There have been no previous studies utilizing diphacinone 50 Bell Labs formula 4 rodenticide to eradicate rats from islands in Hawaii or elsewhere. In preparation for this project new matrix formulations of Bell Labs diphacinone 50 rodenticides were trialed at the NWRC Hawaii Field Station. Bell Labs diphacinone bait formula 4 was shown to be both acceptable and have 100% efficacy against Polynesian rats in two-choice cage feeding trials and was selected for use in this project. The NWRC will take an active operational role by leading the environmental monitoring activities concurrent with the eradication effort. Our role will be similar to that performed by NWRC staff during QA's 1875 and 2441.

A review of the literature was conducted on July 19th 2017 using digitop and google scholar with the following keywords employed singularly or in combination: Lehua Island, Bell Laboratories, diphacinone 50, formula 4 *Rattus exulans*

C. Staff Qualifications

All study participants will 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.

Shane R. Siers, Ph.D., Project Leader, Supervisory Research Wildlife Biologist, will serve as the study director and has 19 years' experience in the care and handling of captive animals and wildlife, to include husbandry, participation in veterinary procedures, field research, and euthanasia, and has led multiple projects assessing rodenticide efficacy in invasive rats and mice in the insular pacific region.

Dean Foster, Biologist, has an MSc in Biology and over 10 years of experience in wildlife science. Mr. Foster has assisted in planning and conducting several invasive mammal research projects, including rodenticide applications and bait fate monitoring, Dean has experience developing and implementing environmental contaminant monitoring programs at federal sites.

Israel Leinbach, M.Sc., Biological Science Technician, has designed and led four large-scale field experiments, and is currently involved in two captive rodent chemical trials within the Hawaii Field Station laboratory facilities. Israel has extensive experience trapping rodents and small animals and collecting environmental samples.

D. Training Assurance

Provide an assurance that participants have read the protocol (especially those who will handle animals), and have completed appropriate training (e.g., CITI or other training – with documentation).

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. Other individuals (volunteers, helpers) assisting with the study will work together with trained staff.

E. Permits

Provide information related to any permits current in possession or being applied for, which are required for the use of animals related to this research activity.

Permitting has been provided by The State of Hawaii - DLNR. A MBTA permit is in process and with be provided before the project begins. The permit will allow NWRC personnel to store and ship samples to Fort Collins, Co. for diphacinone residue analysis of found dead birds

F. Animal Description

1. Animals:
   Fish will be collected and analyzed for rodenticide residues. Species collected will depend on fishing success. The targeted species are as follows:

Achilles Tang	Pākuiku`i	*Acanthurus achilles*
Orange-band Surgeonfish	Na`ena`e	*Acanthurus olivaceous*
Bluestripe Snapper	Taape	*Lutjanus fulvus*
Hawaiian Hog Fish	A`wa	*Bodianus albotaneniatus*

Example near shore gamefish:

Trevally, Jacks	Papio	Carangidae
Goatfish	'Oama	Mullidae
Soldierfish	Menpachi, U'u	Myripristidae
Squirrelfish	'Ala'ihi	*Sargocentron, Neoniphon*
Mackeral scad	'Opelu	*Decapterus macarellus*

Triggerfish (of particular interest due to a historical die-off erroneously attributed to diphacinone exposure):

Black triggerfish	Humuhumu'ele'ele	*Melichthys niger*

Similar fish species not listed here may be sampled.

Any avian species found dead or euthanized by veterinary personnel associated with collaborators (State of Hawaii, Island Conservation, USFWS) will be collected or have tissues harvested and be shipped to the Chemistry Lab Unit in Fort Collins for residue analysis,

2. Species, subspecies (if applicable):
   See above

3. Number and Sex (known or estimated):
   45 fish (15 per three fish type groupings: suspected pellet consumers, triggerfish, and predators)
   Bird mortalities are unpredictable; none are anticipated due to diphacinone use, but natural mortalities may occur – such mortalities will also be assayed to rule out diphacinone intoxication.

   Note: these numbers for larger fish species differ from the numbers in the table under methods, because that table addresses number of samples, not number of fish; larger fish will have two samples (liver and muscle) collected.

4. Additional contingency animals (number and sex):
   Additional animals will be collected in the event fish are found dead near shore during the rat eradication activity on Lehua Island. No fish mortalities are expected with the diphacinone treatment. However, any fish found dead, regardless of apparent cause, will be tested to confirm or rule out diphacinone intoxication.

5. Acceptable Body weight criteria:
   any

6. Acceptable Age criteria:
   any

G. Rationale for involving animals, for appropriateness of species, and for numbers. Provide justification why this study requires the use of animals, and for the numbers to be used.

1. Rationale for involving animals:
   Fish are an important non-target group during island-wide rodenticide applications and are consumed by various user groups visiting Lehua, which makes it necessary to evaluate their toxicity levels.

   Note: Fish sampled during a previous failed eradication attempt on Lehua Island did not contain detectable diphacinone residues.

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

   The species we list above are common at Lehua Island and therefore most likely to be potentially affected by the rodenticide application; they are also representative of the various groups of fish that may be exposed to diphacinone from primary (direct consumption) and/or secondary exposure (consumption of other organisms having ingested bait). Sampling these particular species is necessary to meet our objectives of understanding the levels of rodenticide residues in aquatic biota and potentially pose threat to humans that consume these species.

3. Rationale for numbers of animals to be used, including numbers of animal to be obtained as extra if appropriate (e.g. how many additional animals do you intend to hold in reserve to substitute in for animals found to be unfit for experimentation). Also explain how the numbers of animals requested/planned for relates to the analysis on how numbers were determined or how the numbers requested should satisfy the study requirements.

   The basic sample size for each environmental compartment type is 3 samples per 5 periods, or 15 samples. In a simple simulation power analysis, 1000 iterations of simulated samples of (n = 15) demonstrated 80% power to detect at least one sample positive for diphacinone contamination at a simulated contamination rate of 1.5%. Due to public perception of human health concerns from exposure to toxic residues, we maintain that a high power to detect a low rate of contamination in each environmental compartment justifies this sample size.

H. Source

Describe where the animals will be trapped or obtained, or identify the vendor by name and address.

Free-ranging fish will be collected from wild populations along the shoreline of Lehua Island and opportunistically in the surrounding pelagic waters. Bird carcasses found during searches and ground work will be collected for testing; ill or injured birds euthanized by veterinary personnel associated with collaborators will have tissues or whole carcasses collected and handed over to USDA for shipping to the Chemistry Lab Unit.

I. Method of identification of animals

Explain briefly how animals will be marked or identified to prevent misidentification, and cite any appropriate SOP(s)

Once euthanized, the species, sampling location, date, and weight will be recorded on the outside of the plastic bag with a permanent marking pen. A unique sample ID will be assigned by the Chemistry Lab Unit.

J. Trapping/Collecting

Explain briefly how trapping and collection will be done. As applicable, include the methods to be used and specific procedures such as the frequency of trap checks, removal of animals from traps, specific procedures for extreme temperatures and weather conditions, etc.) and cite any appropriate SOP(s).

The fish will be caught using pole (baited hook and line) or spears.

K. Transport

Explain briefly how transport will be done. As applicable, include the type of vehicle or method of conveyance; temperature control; type, size, and number of cages; numbers of animals per cage; food and water availability; specific procedures for extreme temperatures and weather conditions, total transit time, etc. and cite any appropriate SOP(s).

Samples will be packed in commercially available coolers and kept cold or preferably frozen during storage and shipment to USDA/APHIS/WS/NWRC field station in Hilo, Hawai'i and then on to the NWRC in Fort Collins for analysis. (SOP HS13.03)

L. Handling/restraint

Explain briefly how the animals will be held or restrained (manual vs. chemical) throughout study, and cite any appropriate SOP(s).

Live fish will be euthanized by manually applied blunt force trauma followed by pithing as described in AVMA 2013.

M. Quarantine

Explain briefly the procedure for the quarantine of animals, and cite the appropriate SOP(s).

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

N. Housing/Caging

Explain briefly how housing/caging will be done (including information on feeder animals if used). Provide information regarding special caging or housing requirements, and cite any appropriate SOP(s)

There is no expected need to house animals in this study.

O. Diet/Water

Explain briefly how the animals will be fed and watered, and cite any appropriate SOP(s). Provide information regarding maintenance diets, special diets, and dietary manipulations, and describe components of any test substance formulations.

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

P. Monitoring

Describe how animals will be monitored while on test, especially those who are involved in a toxicity or disease study, or have been injected with a test substance, etc.

There is no expected need to house or monitor animals during this study. Free-ranging wild fish will be collected.

Q. Study End Point:

Describe how the end of the activities which involve the use of animals is determined.

All fish collected for environmental monitoring at Lehua Island will be shipped to the NWRC Hawaii Field Station in Hilo Hawaii and stored frozen until shipped to NWRC headquarters in Fort Collins Colorado for diphacinone residue analysis.

R. Disposition of animals

Address how ill, injured and non-target animals will be handled during the study. Describe the disposition planned for live and dead animals at the end of the study, and cite any appropriate SOP(s).

The fate of dead animals sampled will be their 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.

S. 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: Dr. Thomas Gidlewski

Date of Consultation: July 5th 2017

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

☐ No
☒ Yes - Continue with the following items.

a) Alternative procedures:

Provide a narrative of the sources consulted to determine whether or not alternatives exist to procedures which may cause pain or distress. The narrative should include databases searched or other sources consulted, date of search and years covered by the search, and the keywords and/or search strategy used.

Laboratory analysis of animal carcasses and tissues is a common, standard, and necessary method for determining the toxic residues and heavy metals in the whole-bodies of animals. We know of no other means of accomplishing this purpose.

According to the AVMA 2013 there is no pain or distress expected for sampled fish species due to blunt force stunning prior to pithing nor for crabs and limpets due to anesthetic qualities of chilling or eugenol immersion prior to freezing.

b) Sedatives, analgesics, or anesthetics or Column E Explanation:
Describe the appropriate sedatives, analgesics, anesthetics, or other methods to be used to minimize or alleviate discomfort, distress or pain.

None

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

c) Surgery:
Describe the appropriate provisions for preoperative and postoperative care of animals in accordance with established veterinary, medical, and nursing practices for all activities that involve surgery. No animal will be used in more than one major operative procedure from which it is allowed to recover, unless justified for scientific reasons.

N/A

T. Euthanasia

Describe the appropriate method of euthanasia to be used (cite the current AVMA Guidelines, appropriate SOP, or explain how this will be done). Methods of euthanasia which do not produce rapid unconsciousness and subsequent death, without evidence of pain or distress, must be scientifically justified. (Refer to the current AVMA Guidelines on Euthanasia for approved methods of euthanasia for laboratory and wild animals.)

Live fish will be euthanized by manually applied blunt force trauma followed by pithing as described in AVMA 2013. Crabs and limpets will be anesthetized by chilling on ice and then euthanized by freezing. In the event equipment for freezing is unavailable crabs and limpets will be anesthetized by immersion in a eugenol solution and then euthanized by placing atop ice in a cooler.

U. IACUC Approval

Date of IACUC Approval Letter: July 20, 2017

ENDANGERED SPECIES ACT (ESA) APPENDIX

All activities or programs that are authorized, funded, or carried out, in whole or in part, by federal agencies in the U.S. or upon the high seas are regulated under the ESA. This includes research activities authorized, funded, or conducted by federal agencies and employees.

Before any field activity can take place you must assess the potential effects the proposed action could have on species, populations, or critical habitat protected under the ESA, and then make "effects determinations". Finally, you must maintain an administrative record (i.e., documentation of the evaluation) for the field activity under the ESA.

This appendix will help you document your effects determinations for this action, and determine whether further consultation with the U.S. Fish and Wildlife Service (USFWS) and/or National Marine Fisheries Service (NMFS) is required under section 7 of the ESA.

This appendix does not cover regulatory requirements for state listed species. You must determine those by contacting the State agency responsible for wildlife management.

Links to USFWS/NMFS Resources on Effects Determinations

Effects Determination Guidance (NMFS)
Effects Determination Step-by-Step Instructions (USFWS)
USFWS Consultation Handbook

Effects Determinations Instructions and Decision Tool

1. Is another federal agency taking care of the section 7 responsibilities under ESA for this field activity?
   - ☐ Yes Go to #5, check the box, and follow the instructions.
   - ☒ No Go to #2.

2. **Read all of the instructions under I, II, and III below in order to answer this question!**

   I. Determine the action area, which includes the area where the field activity will actually occur and all areas that reasonably could be directly or indirectly affected by the field activity immediately or in the future.

   II. Go to: USFWS IPaC online planning tool (Hold Ctrl + Click on blue link), click and follow the instructions to map you action area determined in Step I. Then request an "official species list" under "Regulatory Documents" (instructional video; Hold Ctrl + Click on blue link). The official species list will be emailed to you. This official species list will include all species, experimental populations, and critical habitat protected under the ESA that occur in your action area.
   Note: Only consider resources protected under the ESA for this appendix (e.g., do not include species protected under the Migratory Bird Treaty Act or the Bald and Golden Eagle Protection Act).

   III. Based on the results from Step II, do any threatened, endangered, or proposed species (animals and plants), experimental populations, or designated or proposed critical habitat protected under the ESA occur in your action area?
   - ☒ Yes Then go to #3.
   - ☐ No Go to #6, check the box, and follow the instructions.

3. **Read all of the instructions under I, II, and III below in order to properly fill out the table below.**

I. Assess all potential effects of the proposed action **on each** protected species, experimental population, or critical habitat that occurs in your action area by doing the following:

a. Identify all potential stressors resulting from the action to one or more individuals of the species and/or to "primary constituent elements" of the species' habitat; and
   - *Primary constituent elements include: 1) space for individual and population growth, and for normal behavior, 2) food, water, air, light, minerals, or other nutritional or physiological requirements, 3) cover or shelter, 4) sites for breeding, reproduction, rearing of offspring, germination, or seed dispersal, and 5) habitats that are protected from disturbance or are representative of the historic geographic and ecological distributions of a species.*

b. Evaluate all potential pathways in which one or more individuals of the species and/or primary constituent elements of the species' habitat could be exposed to those stressors, including the potential intensity, frequency, and duration of the exposure.

When doing this, you must consider all of the following types of potential effects:
- Direct effects: Changes that occur during implementation of the action.
- Indirect effects: Changes that occur after implementation of the action (at any point in time).
- Interrelated effects: Changes that are the result of a larger action and depend on the larger action for their justification.
- Interdependent effects: Changes that are the result of other actions that would not occur without the action under consideration.
- Cumulative effects: Changes that are the impact of future activities (federal, state, and private) that are reasonably certain to occur after the action has occurred.

II. Then:

A) For the following ESA protection status classifications:
- **Threatened species**
- **Endangered species**
- **Designated critical habitat**
- **Essential experimental population**
- **Non-essential experimental population (inside of a National Park or National Wildlife Refuge)**

a) Determine whether those potential effects are:
   - Zero: No potential for exposure to a stressor.
   - Beneficial: Effects are immediate and wholly positive.
   - Insignificant: Effects relate to the size of the impact and should never reach the scale where "take" occurs. Based on best judgment, a person would not be able to meaningfully measure, detect, or evaluate insignificant effects.
     - *Take includes intentional or incidental harassment, trapping, capture, injury, or death, or otherwise changing the behavior of an individual of a protected species in a way that negatively impacts their fitness, reproduction, or survival, or damaging or altering designated critical habitat.*
   - Discountable: Based on best judgment, a person would not expect these effects to occur, because they are extremely unlikely (this must be justified).
   - Adverse: All other effects are adverse effects. Take must be considered an adverse effect.

b) Identify potential mitigation or conservation measures that can be taken to potentially reduce an adverse effect to an insignificant or discountable effect.
Note: A mitigation measure cannot reduce an insignificant, discountable, or adverse effect to zero effect.

c) Make the appropriate effect determination for the species, experimental population, or critical habitat:

- **No effect (NE)**: The proposed action will have no impact, because there is zero potential for exposure to a stressor resulting from the proposed action (e.g., the species uses completely different habitat units than those directly or indirectly impacted by the action, or is seasonally absent and primary constituent elements of its habitat will not be affected).
    - *Any potential beneficial, insignificant, discountable, or adverse effects of the action means you cannot make an NE determination, even when the potential effects are improbable.*
    - *You also cannot mitigate to an NE determination, but you can move the location of your field activity to another site where the species or critical habitat will have zero exposure to a stressor resulting from the action and then make an NE determination.*
- **May affect, but not likely to adversely affect (NLAA)**: Only applies if the potential effects of the proposed action are wholly beneficial, insignificant, or discountable.
    - *Any potential take resulting from the action means you cannot make an NLTAA determination, even when the take is improbable.*
- **May affect, and is likely to adversely affect (LAA)**: Applies if the proposed action has the potential to cause adverse effects.
    - *You can potentially mitigate to reduce an LAA to an NLAA determination.*

Or:

B) For the following ESA protection status classifications:

- **Proposed species**
- **Proposed critical habitat**
- **Non-essential experimental population (outside of a National Park or National Wildlife Refuge)**

a) Determine whether those potential effects will:

- **Not likely to jeopardize/adversely modify**:
  A) The proposed action is not likely to reduce the reproduction, numbers, or distribution of the proposed species or the non-essential experimental population in a way that would reasonably be expected to directly or indirectly reduce appreciably the likelihood of both the survival and recovery of that species; or
  B) The proposed action is not likely to adversely modify the proposed critical habitat.
- **Likely to jeopardize/adversely modify**:
  A) The proposed action could reasonably be expected to directly or indirectly appreciably reduce the likelihood of both the survival and recovery of the proposed species or the non-essential experimental population by reducing reproduction, numbers, or the distribution of that species; or
  B) The proposed action is likely to adversely modify the proposed critical habitat.

III. Finally, **for each ESA-protected resource** record in the table below: **a)** the name, **b)** the protection status, **c)** the appropriate effect determination, and **d)** an explanation/rationale/justification for the effect determination for each species (including mitigation measures, if applicable), experimental population, or critical habitat in your action area. Archive all supporting documentation (e.g., USFWS informational resources, peer-reviewed publications, survey data). Once you have completed the table, go to #4.

a. Name of species/experimental population/critical habitat:

Hawaiian monk seal *Neomonachus schauinslandi*

Select the species' ESA protection status and your effect determination below (complete only one column of this section)

b. ESA protection status:

☐ Threatened species
☒ Endangered species
☐ Designated critical habitat
☐ Experimental population (check which one applies below):
- ☐ Essential
- ☐ Non-essential, inside a National Park or Refuge

c. Effect determination

☒ NE *(Note: you cannot mitigate to an NE)*
☐ NLAA *(check all that apply below)*
- All potential effects are either:
  - ☐ Beneficial Effects
  - ☐ Insignificant Effects
  - ☐ Discountable Effects

☐ LAA

b. ESA protection status:

☐ Proposed species
☐ Proposed critical habitat
☐ Experimental population
- ☐ Non-essential, outside of a National Park or Refuge

c. Effect determination:

☐ Not likely to jeopardize/adversely modify
☐ Likely to jeopardize/adversely modify

d. Explanation/rationale/justification for effect determination, including mitigation measures, if applicable:

USDA personnel will be collecting nearshore fish, crabs, and limpets on Lehua Island in conjunction with a Polynesian rat eradication action being undertaken by the state of Hawaii under an EA that was written by the local USFWS office. The FWS EA stipulates eradication and environmental sampling activities will maintain a 100m buffer from seals and turtles that have landed on the shore and USDA personnel will be required to meet this requirement. Sampling activities will take place when either no seals are nearby or the sampling site will be moved away from seals and thus seals will have zero exposure to the proposed sampling. Hawaiian monk seals forage for food far offshore and thus nearshore fish sampling will not effect their food supply.

a. Name of species/experimental population/critical habitat:

Green sea turtle *Chelonia mydas*

Select the species' ESA protection status and your effect determination below (complete only one column of this section)

b. ESA protection status:

☒ Threatened species
☐ Endangered species
☐ Designated critical habitat
☐ Experimental population (check which one applies below):
- ☐ Essential
- ☐ Non-essential, inside a National Park or Refuge

c. Effect determination

☒ NE *(Note: you cannot mitigate to an NE)*
☐ NLAA *(check all that apply below)*
- All potential effects are either:
  - ☐ Beneficial Effects
  - ☐ Insignificant Effects
  - ☐ Discountable Effects

☐ LAA

b. ESA protection status:

☐ Proposed species
☐ Proposed critical habitat
☐ Experimental population
- ☐ Non-essential, outside of a National Park or Refuge

c. Effect determination:

☐ Not likely to jeopardize/adversely modify
☐ Likely to jeopardize/adversely modify

d. Explanation/rationale/justification for effect determination, including mitigation measures, if applicable:

USDA personnel will be collecting nearshore fish, crabs, and limpets on Lehua Island in conjunction with a Polynesian rat eradication action being undertaken by the state of Hawaii under an EA that was written by the local USFWS office. The FWS EA stipulates eradication and environmental sampling activities will maintain a 100m buffer from seals and turtles that have landed on the shore and USDA personnel will be required to meet this requirement. Sampling activities will take place when either no sea turtles are nearby or the sampling site will be moved away from sea turtles and thus seals will have zero exposure to the proposed sampling.

a. Name of species/experimental population/critical habitat:

Newell's Shearwater Puffinus auricularis newelli

Select the species' ESA protection status and your effect determination below (complete only one column of this section)

b. ESA protection status:

☒ Threatened species
☐ Endangered species
☐ Designated critical habitat
☐ Experimental population (check which one applies below):
  ☐ Essential
  ☐ Non-essential, inside a National Park or Refuge

c. Effect determination

☒ NE *(Note: you cannot mitigate to an NE)*
☐ NLAA *(check all that apply below)*
  All potential effects are either:
  ☐ Beneficial Effects
  ☐ Insignificant Effects
  ☐ Discountable Effects
☐ LAA

b. ESA protection status:

☐ Proposed species
☐ Proposed critical habitat
☐ Experimental population
  ☐ Non-essential, outside of a National Park or Refuge

c. Effect determination:

☐ Not likely to jeopardize/adversely modify
☐ Likely to jeopardize/adversely modify

d. Explanation/rationale/justification for effect determination, including mitigation measures, if applicable:

Newell's shearwater are suspected to breed on Lehua Island at high elevations and on cliffs. All listed seabirds that may nest on Lehua Island forage out at sea over deep pelagic waters and do not consume near shore fish and invertebrates. Thus our sampling of near shore fish and intertidal invertebrates will be in locations where shearwaters will not be found and so our activities will not impact shearwaters that may be on Lehua Island.

a. Name of species/experimental population/critical habitat:

Hawaiian Petrel *Pterodroma sandwichensis*

Select the species' ESA protection status and your effect determination below (complete only one column of this section)

b. ESA protection status:
☐ Threatened species
☒ Endangered species
☐ Designated critical habitat
☐ Experimental population (check which one applies below):
☐ Essential
☐ Non-essential, inside a National Park or Refuge

c. Effect determination
☒ NE *(Note: you cannot mitigate to an NE)*
☐ NLAA *(check all that apply below)*
All potential effects are either:
☐ Beneficial Effects
☐ Insignificant Effects
☐ Discountable Effects
☐ LAA

b. ESA protection status:
☐ Proposed species
☐ Proposed critical habitat
☐ Experimental population
☐ Non-essential, outside of a National Park or Refuge

c. Effect determination:
☐ Not likely to jeopardize/adversely modify
☐ Likely to jeopardize/adversely modify

d. Explanation/rationale/justification for effect determination, including mitigation measures, if applicable:
Hawaiian petrel are suspected to breed on Lehua Island at high elevations and on cliffs. All listed seabirds that may nest on Lehua Island forage out at sea over deep pelagic waters and do not consume near shore fish and invertebrates. Thus our sampling of near shore fish and intertidal invertebrates will be in locations where petrels will not be found and so our activities will not impact Hawaiian petrels that may be on Lehua Island

a. Name of species/experimental population/critical habitat:
Band-rumped Storm-petrel *(Oceanodroma castro*

Select the species' ESA protection status and your effect determination below (complete only one column of this section)

b. ESA protection status:
☐ Threatened species
☒ Endangered species
☐ Designated critical habitat
☐ Experimental population (check which one applies below):
☐ Essential
☐ Non-essential, inside a National Park or Refuge

c. Effect determination
☒ NE *(Note: you cannot mitigate to an NE)*
☐ NLAA *(check all that apply below)*
All potential effects are either:
☐ Beneficial Effects
☐ Insignificant Effects
☐ Discountable Effects
☐ LAA

b. ESA protection status:
☐ Proposed species
☐ Proposed critical habitat
☐ Experimental population
☐ Non-essential, outside of a National Park or Refuge

c. Effect determination:
☐ Not likely to jeopardize/adversely modify
☐ Likely to jeopardize/adversely modify

d. Explanation/rationale/justification for effect determination, including mitigation measures, if applicable:
Band-rumped storm-petrel are suspected to breed on Lehua Island at high elevations and on cliffs. All listed seabirds that may nest on Lehua Island forage out at sea over deep pelagic waters and do not consume near shore fish and invertebrates. Thus our sampling of near shore fish and intertidal invertebrates will be in locations where storm-petrels will not be found and so our activities will not impact band-rumped storm-petrel that may be on Lehua Island.

Note: To add species, experimental populations, or critical habitat: 1) click anywhere in the table cells above, and then 2) click the "+" in the bottom right corner of the cells selected.

4. Once you have completed the table above, select the appropriate option below:
☒ All species, experimental populations, and critical habitat effect determinations are NE or "Not likely to jeopardize/adversely modify". Go to #6, check the box, and follow the instructions.
☐ One or more species, experimental populations, or critical habitat effect determinations are NLAA, and none of the determinations are LAA or "Likely to jeopardize/adversely modify". Go to #7, check the box, and follow the instructions.
☐ One or more species or critical habitat effect determinations are LAA or "Likely to jeopardize/adversely modify". Go to #8, check the box, and follow the instructions.

ESA Appendix Conclusion

5. ☐ Another federal agency is fulfilling the section 7 responsibilities for this proposed action.
   - Do not conduct the requested field activities until no effect determinations have been made by the other agency or consultation/conference with USFWS/NMFS is complete. You must be informed of and follow the requirements of the consultation/conference.
   - **You are finished with the ESA Appendix and your responsibilities under the ESA unless an additional species or critical habitat is protected under the ESA in the action area during the action or if the action area expands.**

6. ☒ A **no effect** or **not likely to jeopardize/adversely modify** determination is made for all species, experimental populations, and critical habitat protected under the ESA for the proposed action.
   - Save and archive your official species list and any other information used to reach this conclusion.
   - **You are finished with the ESA Appendix and your responsibilities under the ESA unless an additional species or critical habitat is protected under the ESA in the action area during the action or if the action area expands.**

7. ☐ The proposed action is **may affect, but is not likely to adversely affect** one or more species, experimental populations, or critical habitat protected under the ESA within the action area.
   - The NWRC NEPA contact will initiate the informal consultation process with USFWS/NMFS Ecological Services. **Written concurrence from USFWS/NMFS Ecological Services on the NLAA determination(s) is required before the action may be undertaken, or before an irreversible or irretrievable federal commitment to the action is made.** Correspondence from USFWS Refuge personnel will not suffice. This process usually takes at least 1 month.
   - Save and archive all documents and correspondence, including the official species list and concurrence letter from USFWS/NMFS.
   - **You are finished with the ESA Appendix, but not with your responsibilities under the ESA.**

8. ☐ The proposed action **may affect, and is likely to adversely affect** or one or more species, experimental populations, or critical habitat within the action area, and/or is **likely to jeopardize** the continued existence of proposed species or experimental populations, and/or is **likely to adversely modify** proposed critical habitat.
   - Contact the NWRC NEPA contact to initiate a formal consultation and conference process with USFWS/NMFS Ecological Services. **The formal consultation must be concluded before the action may be undertaken, or**

before an irreversible or irretrievable federal commitment to the action is made. This process takes a minimum of 6 months.

- Save and archive all documents and correspondence, including the official species list, the Biological Assessment, Section 10 permits (if applicable), and the Biological Opinion from USFWS/NMFS.
- **You are finished with the ESA Appendix, but not with your responsibilities under the ESA.**

NATIONAL ENVIRONMENTAL POLICY ACT (NEPA) APPENDIX

This appendix is intended to aid the Study Director with determining whether a proposed project qualifies for a categorical exclusion as allowed by the USDA APHIS Implementing Regulations (7 CFR, part 372). Categorical exclusions are classes of federal actions that do not individually or cumulatively have a significant effect on the human environment.

- **Complete the Endangered Species Act (ESA) Appendix prior to completing this appendix.**
- **This appendix does not cover regulatory requirements for States. You must determine those by contacting the appropriate State agency.**

A. Is another agency completing the NEPA and ESA requirements for the proposed action, and do they adequately address all proposed NWRC activities?

☒ Yes – Please contact the NWRC NEPA Contact to determine the appropriate level of documentation. (A copy of the document must be included when your study is archived).

The USFWS has prepared an Environmental Assessment (EA) and issued a Finding of No Significant Impact (FONSI) for their actions. The NWRC is listed as a cooperator on the EA and our actions are described in Appendix E of the EA and in further detail within this protocol. NWRC has evaluated the effects of the proposed monitoring activities and has determined that a Categorical Exclusion (CatEx) is appropriate.

References:
- Final Environmental Assessment Lehua Island Ecosystem Restoration Project – July 2017
- Finding of No Significant Impact for the Proposed Lehua Island Ecosystem Restoration Project By The U.S. Fish and Wildlife Service Kauai Kounty, Hawaii – signed July 6, 2017

☐ No – Continue to question B.

B. What was your conclusion in the ESA Appendix?

☐ The proposed action will require a formal consultation with USFWS or the National Marine Fisheries Service (NMFS) – This study does not qualify for a Categorical Exclusion, and an EA or EIS should be prepared before initiation of the project. You are done with this appendix. Contact the NEPA Coordinator for assistance.

☐ The proposed action will require an informal consultation with USFWS or NMFS – This study may qualify for a Categorical Exclusion if you determined that the proposed action may affect, but is not likely to adversely affect all listed species, experimental populations, or critical habitats **AND** USFWS or NMFS concurs in writing. – Continue to question C.

☒ No consultation (formal or informal) with USFWS or NMFS is required under the ESA – Continue to question C.

C. Do any agency actions classified as undertakings under the National Historical Preservation Act (NHPA) result in adverse effects to historic properties within the area of potential effects (http://www.achp.gov/flowexplain.html).

Undertakings are projects, activities or programs either funded, permitted, licensed or approved by a Federal Agency. Undertakings may take place either on or off federally controlled property and include new and continuing projects, activities, or programs and any of their elements not previously considered under Section 106 of the NHPA.

Adverse Effects occur when an undertaking may directly or indirectly alter characteristics of a historic property that qualify it for inclusion in the Register. Examples of adverse effects include physical destruction or damage; alteration not consistent with the Secretary of the Interior's *Standards*; relocation of a property; change of use or

physical features of a property's setting; visual, atmospheric, or audible intrusions; neglect resulting in deterioration; or transfer, lease, or sale of a property out of Federal ownership or control without adequate protections.

Use one of the following links to determine if historical properties fall within the proposed action area:

a. https://www.nps.gov/maps/full.html?mapId=7ad17cc9-b808-4ff8-a2f9-a99909164466 (Useful for smaller geographic areas)
b. http://nepassisttool.epa.gov/nepassist/entry.aspx (Useful for larger geographic areas)

☐ Yes – Contact the State Historic Preservation Office (SHPO) for consultation (http://ncshpo.org/shpodirectory.shtml). This study may not qualify for a Categorical Exclusion and an EA or EIS may need to be prepared before initiation of the project if there are concerns from the SHPO. (A copy of the letter to the SHPO and any other information regarding the consultation must be included when your study is archived). – Continue to question D.

☒ No – Continue to question D.

D. Do any agency actions occur on tribal lands or ceded tribal lands? Use the following link to determine if tribal lands fall within the proposed action area:

a. http://www.arcgis.com/home/webmap/viewer.html?webmap=2a19e6ffe6934e09aaa0fa82f1bc0148

☐ Yes – Contact the WS State Director and WS tribal liaison to determine if there is a need for formal consultation on the program/study. This study may not qualify for a Categorical Exclusion and an EA or EIS may need to be prepared before initiation of the project if there are any tribal concerns. (A copy of the tribal letter must be included when your study is archived). – Continue to question E.

☒ No – Continue to question E.

E. Is the study a routine measures activity, such as identification, surveying, testing, removals, control, and sampling that will not cause physical alteration of the environment?

☒ Yes – You must be able to check all the below boxes and provide justification (if you are unable to check all the boxes, you must check "No") - Continue to question F.

☒ 1. Be localized or contained in areas where people are not likely to be exposed, and is limited in terms of quantity

☒ 2. Does not cause contaminants to enter water bodies (this includes runoff, drift or volatilization)

☒ 3. Does not cause bioaccumulation (the accumulation of a toxicant at a rate faster than it can be metabolized or excreted from an organism. In aquatic systems the bioconcentration factor (BCF) can be used to determine the potential for bioaccumulation. The octanol water partition coefficient (Kow) can also be used to determine the potential for bioaccumulation in aquatic and terrestrial organisms).

☒ 4. No extraordinary circumstances identified (adverse effects to environmentally sensitive areas or resources, or public controversy over the environmental effects of the proposed action)

NWRC staff will be collecting environmental samples and is a routine measures activity. We will not be applying toxicants. Our role in this rodenticide application is only to sample and test soil, water, fish, and aquatic inverts for the presence of rodenticide residues.

☐ No – Based on the information provided above this study does not qualify for a Categorical Exclusion and an EA or EIS should be prepared before initiation of the project. You are done with this appendix. Contact the NEPA Coordinator for assistance.

F. Summarize the risk to each group in the below with consideration of effects and the potential for exposure individually, and in relation to other impacts that may occur in the study area. Provide a justification for each endpoint and check the appropriate box below.

> ***Cumulative impacts*** are impacts on the environment which results from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency (Federal of non-Federal) or person undertakes such other actions. Cumulative impacts can result in individually minor but collectively significant actions taking place over a period of time.

1. Risk to human health
2. Risk to target species
3. Risk to non-target species

There is no risk to human health or non-target species. If non-target fish are caught by hook and line they will be released unharmed. There is no cumulative impacts risk to targeted fish and invertebrate species through our sampling and analysis of targeted species tissues. The collected species are common, available to the general public for consumptive harvest, we will only collect a small number, and this is not a long term monitoring project with extensive sampling.

Does this activity pose a risk to human health or target and non-target species (including cumulative impacts) that will not be minimized or mitigated?

☐ Yes – Based on the information provided above this study does not qualify for a Categorical Exclusion and an EA or EIS should be prepared before initiation of the project. You are done with this appendix. Contact the NEPA Coordinator for assistance.

☒ No – Continue to question G.

G. Will this study have a disproportionate adverse effect to children, minorities and low income populations? (Use the information under letter F (Risk to human health) and the location of the proposed study (i.e., potential for exposure) to discuss whether there would be any disproportionate impacts to children, minorities, and low income populations).

No people live on Lehua Island and the species of fish and invertebrates we will be collecting are common and numerous. Our sampling efforts will not significantly reduce the local populations of any species that may be targeted by local peoples for subsistence or commercial fishing.

☐ Yes – Based on the information provided above this study does not qualify for a Categorical Exclusion and an EA or EIS should be prepared before initiation of the project. You are done with this appendix. Contact the NEPA Coordinator for assistance.

☒ No – The study meets the criteria for Categorical Exclusion - No further action is needed for NEPA.