DRAFT Mouse Biomarker Trial: South Farallon Islands

Trials to Examine Bait Consumption and Non-Target Exposure Risk

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1. INTRODUCTION

1.1 Purpose

The Farallon Islands provide critical habitat for seabirds and pinnipeds, and support some of the world's largest nesting seabird colonies including Ashy Storm-Petrel (*Oceanodroma homochroa*), Brandt's Cormorant (*Phalacrocorax penicillatus*) and Western Gull (*Larus occidentalis*). On the South Farallon Islands, which include two main islands - Southeast Farallon and West End Island, introduced house mice (*Mus musculus*) appear to be directly and indirectly impacting the breeding success of burrow nesting seabirds (Ainley and Boekelhide 1990; Sydemann et al. 1998; Pyle 2001).

The US Fish and Wildlife Service (USFWS) propose to eradicate house mice from the South Farallon Islands to improve habitat for nesting seabirds. The preferred technique for mouse eradication requires an aerial application of pelletized grain bait containing rodenticide across the island. Further background information and justification for this eradication are discussed in Howald et al. (2003) and the environmental impacts of this proposed action are analyzed in a draft environmental assessment (EA).

Removal of mice will result in biodiversity benefits for native flora and fauna on the Farallon Islands. Eradicating house mice from islands, however, has proven to be more challenging than removing rats from islands. While rats have been eradicated from over 318 islands worldwide, only approximately 30 successful mouse eradications have been documented (Howald et al. 2007). Furthermore, the failure rate of rat eradications is reported at 5percent, while in mouse eradications it is 19 to 38 percent (Howald et al. 2007; MacKay et al. 2007). Despite a global review of mouse eradication attempts, no consistent explanations have been identified for this low rate of success. However, compared to rats, it is known that mice are less susceptible to some rodenticides, have a smaller home range and a more complex social structure, and feed somewhat sporadically from several locations rather than foraging on a regular, reliable food source (Macdonald and Fenn 1994). The behavioral and foraging differences between rats and mice indicate that to successfully remove mice from islands, a high standard of bait quality and availability to mice must be guaranteed. These standards can be better achieved by implementing anon-toxic trial bait application prior to the proposed eradication to indicate any site-specific differences in mouse behavior, densities, or bait acceptability, which will help to refine standardized eradication methods for the local environment.

Prior to the proposed eradication, a field trial will be conducted to: i) validate the acceptability of bait and persistence of the biomarker by house mice; ii) determine the rate of bait removal to test the target application rate for the eradication; iii) determine the probability of eradication by using mice exposed to a biomarker from a non-toxic, biomarker-infused bait applied at the target application rate; and iv) to evaluate what non-target species are at risk of rodenticide exposure during an eradication operation.

This proposal describes a field trial that will be conducted in November 2010 to assess the efficacy of mouse eradication at the target application rate of a preferred bait (using a placebo replica infused with the non-toxic biomarker pyranine), and to monitor non-target species exposure to broadcast pellets.

1.2 Objectives

The field trials on Southeast Farallon Island this fall are proposed to evaluate key factors related to the eradication to best inform a plan proceeding with a high likelihood of success. Specifically, the trial will be designed to address the following questions:

i) At what density and phase of the reproductive cycle are mice during the proposed time period (fall) on the Farallon Islands?

ii) Do mice demonstrate a preference for a non-toxic replica of the preferred bait, Brodifacoum-25D Conservation, to be used in the proposed eradication compared to naturally available food resources?

iii) How many days after exposure to bait is the biomarker detectable in mice?

iv) Based on rates of removal, at what density can bait be applied to persist for the duration of the target exposure window (10 days) for mice on the island?

v) At the target application rate will bait be delivered to all mice within the study areas?

vi) What non-target species are at risk of rodenticide exposure during an eradication operation, and at what rates might they be exposed to or consume the bait directly or through secondary or indirect contact?

1.3 Impacts of House Mice to Island Ecosystems

The house mouse is among the most widespread of all mammals, a result of its close association with humans and the relative ease with which it can be transported and introduced to new locations. House mice are among the vertebrates considered to be "significant invasive species" on islands of the South Pacific and Hawaii, having probably reached all inhabited islands in the Pacific as well as some uninhabited islands (Atkinson and Atkinson 2000). The resourcefulness of house mice is evident from their global distribution and broad habitat range including buildings, agricultural land, coastal regions, grasslands, salt marshes, deserts, forests and subantarctic areas (Efford et al. 1988; Triggs 1991; Atkinson and Atkinson 2000).

House mice eat a variety of seeds, fungi, insects, other small animals, reptiles and eggs of small birds, and their diet directly contributes to ecosystem-wide perturbations including effects on native fauna and flora (Rowe-Rowe et al. 1989; Crafford 1990; Amarasekare 1994; Cole et al. 2000). For example, Newman (1994) found that increased predation by house mice caused the capture rate for McGregor's skink (*Cyclodina macgregori*) to decline on Mana Island, New Zealand. After successful mouse eradication, the population of McGregor's skink, the gecko (*Hoplodactylus maculatus*), and the endemic giant cricket (*Deinacrida rugosa*) increased significantly. More recently, on Gough Island in the South Atlantic, house mice have been documented depredating large nestlings of Tristan Albatross *Diomedea dabbanena* and Atlantic Petrels *Pterodroma incerta*, and causing significantly reduced nesting success in these species (Wanless et al. 2007).

1.4 Project Setting

The Farallon Islands are a small group of five islands located 51 km (32 miles) west of San Francisco. The total land area is 83 ha (211 acres) of which two islands, Southeast Farallon (31 ha) and West End, comprise the majority (44 ha). West End and Southeast Farallon Island (SEFI) are separated by a narrow gap known as the Jordan Channel. The islands are projections of a granitic ridge that rise from the sea floor, and the highest point is on SEFI at 105 m (343 ft). The islands are home to the largest seabird breeding colony in the continental U.S.

During the 1800s, the Farallon Islands were exploited by seal hunters and egg collectors providing major sources of food for nearby San Francisco. In 1855 the U.S. Coast Guard established a lighthouse on SEFI and subsequently several keepers, their families and domestic animals lived there until 1965. It is likely that the house mouse was introduced to SEFI in the 1800s, along with domestic cats and rabbits (*Oryctolagus cuniculus*) during later settlement by lighthouse staff. The rabbits and cats were removed in 1974 but the house mice remain.

Owing to the overexploitation of the islands, in 1909 the North Farallon Islands were designated a national wildlife refuge (the Farallon Reservation), and in 1969 the South Farallon Islands were included to create the Farallon National Wildlife Refuge. The islands are managed by the U.S. Fish and Wildlife Service, in conjunction with the PRBO Conservation Science and formerly with the U.S. Coast Guard. The surrounding submerged lands are part of the recently established Gulf of the Farallones National Marine Sanctuary, which is administered by the National Marine Fisheries Service.

1.5 Summary of Knowledge of House Mice on the Farallon Islands

Over the past 200 years, the South Farallon Islands supported introduced rabbits, cats and house mice. Like rabbits and cats (which were successfully eradicated), house mice were introduced by previous human occupants of the island – before it became part of the Farallon National Wildlife Refuge in 1969. Information collected to date on the house mouse of the South Farallon indicates they:

1. Are distributed relatively evenly on Southeast Farallon Island (SEFI) and have been observed on the West End Island (Irwin 2006).
2. Have not been observed on other islands in the chain (e.g., North or Middle Farallon Islands), nor are they suspected to occur on these islands, which have no history of human occupation.
3. Breed prolifically from April through October, and die off in equally large numbers from November through April (Irwin 2006).
4. Feed on native plants, invertebrates and seabirds (Jones and Golightly 2006; Irwin 2006; Ainley and Boekelheide 1990).

Trap studies carried out at 28 sites between 2001 and 2003 on SEFI suggest that mouse numbers peak between September and December reflected by a peak in trapping success (71%) in October, after which they decline to a low of only 2% trapping success in April-May (Irwin 2006). During these three years, average trapping success over the entire year was relatively low (25%-41%), a mean trap rate of 31%.

2. METHODS

2.1 Sensitive Species and Habitats Avoidance

Prior to arriving at Farallon Island, the three Island Conservation staff accompanying the Project Manager will receive education and training regarding the sensitive and listed resources on the island, including which areas can and cannot be traversed on foot without disturbing sensitive seabird burrows or hauled out pinnipeds. To facilitate ease of implementation of the trial, study areas have been selected that are a relatively flat and open so as to avoid any harassment of wildlife or impacts to nesting habitat in the non-season. In addition to this training, IC will receive a second on-site orientation briefing of sensitive resource avoidance and safety procedures by the Refuge Manager and/or PRBO or IC Project Manager. IC personnel conducting this trial will include Dan Grout, Jake Bonham, Maddy Pott and Erik Oberg. The specific methods detailed below may require amending on the island as the protocols are tested, but the intent is to provide those types of information useful for assessing the proposed efficacy and potential non-target impacts of an eradication, as described in the Cooperative Agreement between the USFWS and Island Conservation signed on September 23 of this year (USFWS 2010).

2.2 Selection of the Biomarker Study Areas

The primary considerations for study sites for the bait trials are:

- Areas that have a comparatively high density of mice
- Areas that have a comparatively high density of roosting gulls (which are presumed at present to be the most likely primary non-target bait consumer)
- Areas that can be traversed relatively easily on foot without disturbing sensitive resources such as seabird burrows or pinnipeds. To facilitate ease of implementation, a relatively flat and open area is required for treatment.
- Areas that provide sufficient ground area to provide meaningful bait uptake results from two "core" trapping areas within a large baited "buffer" zone.

After a one-day preliminary study site investigation on October 13, 2010, the area areas identified on Southeast Farallon that appear to fit these criteria best are: 1) the Marine Terrace area for the bait trial, and 2) North Landing for the uptake calibration trial. These proposed areas were inspected and

determined to be suitable after discussions on site with FWS Refuge Manager and PRBO staff on measures and adjustments to reduce impact to sensitive bird and pinniped areas. The approximate study areas are mapped in Figures 1 and 2.

2.3 Mouse Indices of Abundance

Indices of abundance (IOA) and density estimates can be useful variables to assess, as they can assist in confirming where the mouse population is increasing or decreasing in its annual cycle, which greatly affects the density of bait required to reach every mouse. Assessing whether the mouse population is rising or falling from month to month is a very important factor when considering when to initiate eradication efforts. Indices of abundance (IOA) for mice will be assessed utilizing two different methods.

2.3.1 Irwin's Index of Abundance

This index is an effort to provide some longitudinal data by employed repeated constant-effort trapping on a monthly basis to gauge the annual population cycles of the mouse. This program will replicate the IOA trapping program formerly conducted on the island from 2001to 2004 (Irwin 2006). The prior mouse trapping survey consisted of assessing relative mouse abundance over three years by snap-trapping monthly at 28 stratified random sampling points. These 28 trap locations consisted of seven trap locations along four different transects, with each of the four transects sampling a different habitat strata (Figure 1). These transects sampled all habitat types on the island including steep hill-sides, flat ground, native vegetation dominated, non-native dominated, and areas close to and far from the shoreline.

These 28 trap locations will be trapped for three nights each month beginning in November 2010 and will continue to be trapped monthly by PRBO/USFWS staff/interns for the next year until implementation. While the traps at the 28 locations to be surveyed in this 2010-2011 study will be live-traps (not the snap-traps used in the previous surveys) it is felt that the data will still provide some useful comparisons of trap success rates, and may provide some longitudinal data to compare to past population cycles. Trap success and unique individuals will be recorded during a three-day trapping session using 9-inch Sherman live traps (H.B. Sherman Traps, Tallahassee, Florida). Captured animals will be euthanized following AVMA guidelines, and representative animals will have tail tissue preserved for DNA analysis.

2.3.2 Index of Abundance Trapping Grid

A second index will also be developed utilizing a one-time trapping grid 0.25 hectares in size (50m x 50m). The trap grid location will be in the Marine Terrace area between the two core baiting grids (Figure 2). The 100 Sherman live-traps traps arranged in a 10 x 10 grid with traps spaced at 5m intervals. Traps will be lined with a small handful of polyester fiberfill for thermal protection and baited in the evening with whole oats or peanut butter mixed with rolled oats. Traps will be checked each morning at dawn.

Captured mice will be tagged using Size 1 (National Band and Tag Co., Newport, KY) ear tags, and age class, sex, weight, and reproductive status determined. Each captured mouse will be released unharmed at the point of capture. For each mouse recaptured during subsequent trap sessions, the capture station and mouse ear tag number will be recorded. A minimum of five consecutive trap nights will be conducted. A rough estimate of mouse density will be approximated using the program CAPTURE or similar program.

Capture rates will be calculated as (R) per trap nights (TN) (defined as one trap set per night) and corrected (C) by 1/2 for all sprung traps without a mouse catch (Nelson and Clark 1973) and notated as R/C *TN. This requires a trap status for each trap checked in the field as either “sprung” or “unsprung”. For “sprung” traps, evidence of a mouse capture or no obvious signs of capture will be recorded.
The marking and release of these mice could also inform the results of the biomarker mouse trapping to be conducted at the two core trapping grids, should any of these ear-tagged mice appear in subsequent trapping efforts (See Section 2.4.5).

2.4 Bait Acceptability and Biomarker Persistence

Acceptability of the bait to be used, its detectability and the persistence of the biomarker will be evaluated in laboratory trials on the island just prior to the baiting trial in the field.

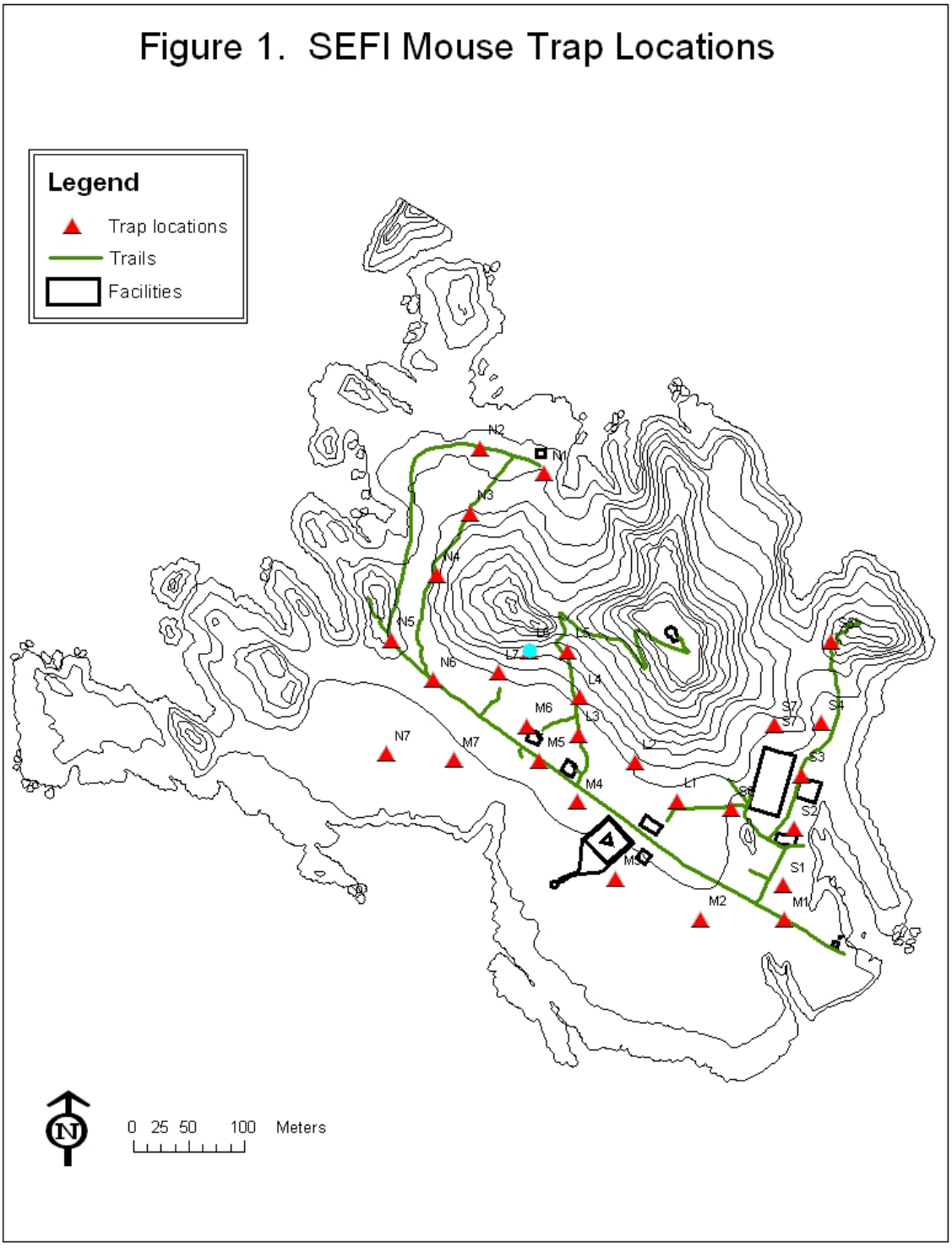
Figure 1. SEFI Mouse Trap Locations
Legend
Trap locations
Trails
Facilities
N1
N2
N3
N4
N5
N6
N7
M1
M2
M3
M4
M5
M6
M7
L1
L2
L3
L4
L5
L6
L7
S1
S2
S3
S4
S5
S6
S7
N
0 25 50 100 Meters

2.4.1 Biomarker bait

The bait used during the bait consumption study will be a non-toxic replica of Brodifacoum-25D bait (Bell Laboratories Inc., Madison, WI, EPA Reg. No. 56228-37). 25D is a cereal-grain bait colored green and compressed into 3/8" diameter pellets. The 25D bait trail formulation is an exact mimic of the preferred toxic bait to be used in the proposed eradication except it is infused with the non-toxic biomarker dye pyranine. Pyranine (also known as Solvent Green 7) is a commonly used water-soluble dye found in commercially available products such as fluorescent marker pens, shampoos, soaps and cosmetics. It is also used to trace water-flows such as in plumbing systems, sewers and natural water-courses. It is non-hazardous, non-flammable and is not regulated as a hazardous material (refer to Appendix 1 for pyranine MSDS). Pyranine's most useful attribute for this and similar studies is that fluoresces green under ultra-violet light. During bait palatability trials for *Rattus* sp. and *Mus* sp. on Macquarie Island (Australia) in 2005, pyranine was used in a cereal-based placebo bait, and under ultra-violet light was detected in animals' mouths, paws, anus and fur, as well as in feces and urine (K. Springer pers. comm.). Pyranine has also been used as a biomarker in trial rat eradications in New Zealand to investigate potential bait uptake in non-target birds on Macauley Island (Greene et. al 2002) and Little Barrier Island (Greene and Dilks 2004).

2.4.2 Bait Acceptability

While there is limited data available in the published and gray literature in of the U.S., New Zealand and Australia as to bait size acceptability in mice, mice reportedly demonstrate no significant preference for bait size (Wilkinson, pers. com). A 3/8" diameter non-toxic replica pellet of the preferred bait to be used during the proposed eradication will be tested for palatability in lab trials on the island trials. Test bait will be provided to a group of mice as well as naturally available food sources (*Claytonia* spp. or *Hordeum* spp. green plant material, and Coleopteran beetle or larvae) (Jones and Golightly 2006). Individual mice will be live-trapped and held in a field laboratory for ~24 hours pre-trial. Each mouse will be presented with paired food choices placed in random locations within the cage to reduce spatial selectivity. Trials will be scored by first bait/food type selected, with observations made for an additional period after choice to determine if animals switched to consuming the alternative bait/food type. Summary statistics of mouse preference for bait size and food type will be quantified.

2.4.3 Biomarker Detection and Persistence Assay

A lab detection assay will be conducted on island prior to field-based biomarker trials to index detection level and persistence of pyranine sign in mice. These trials will be conducted on island during the first week at the beginning of the field trial. Fourteen mice will be individually housed in plastic cages with wire lids and given polyfill fiber for thermal protection/bedding. Mice will be acclimatized to the laboratory environment for ~24 hours, during which they will have free access to standard rodent feed.

In the pyranine retention study, mice will be randomly assigned to one of three non-toxic, biomarker bait exposure groups: <LD50, >LD50, and control. LD50 is defined as the amount of rodenticide bait that must be consumed in order to reach roughly 50% probability of mortality. House mice require approximately 1- 2.6% of their bodyweight in a single dose to achieve acute oral toxicity of brodifacoum delivered at 20 ppm (Eason and Ogilvie 2009). Each bait exposure group will contain 4 mice, with a control treatment group containing two mice.

Mice in each group will receive a single feeding of a set number of non-toxic Brodifcaoum 25D Conservation bait pellets (infused with the biomarker pyranine; Bell Laboratories, Madison, WI) to achieve the assigned exposure. The number of pellets will be determined following validation of mean pellet mass (measured in grams), however should approximate 0, 1, 2 and 4 pellets, respectively. Pellets will be placed inside the cage. Mice in the control group will be offered standard rodent feed. After all bait pellets are consumed by each mouse, they will be maintained on a diet of standard rodent feed. All mice in each group will be inspected externally every 24 hours for signs of the biomarker on the anus, tail and mouth. Mice no longer exhibiting external exposure to biomarker will be euthanized according to current UVMA standards and an internal examination of the alimentary tract (including stomach, intestines, and ceacum) will be conducted for fluorescence using handheld ultraviolet lights. For each mouse examined the intensity of fluorescence will be qualitatively assigned to a rating scale.

Score	Description
-	No fluorescence detected
+	Fluorescence detected in limited intensity and/or area covered
++	Fluorescence detected in moderate intensity and/or area covered
+++	Fluorescence detected in high intensity and/or area covered

At termination all basic morphometric information will be collected for each mouse, including body measurements, sex, and weight. For each exposure group the proportion of mice displaying fluorescence will be calculated for each sampling period. Maximum retention will be defined as the number of days post-feeding where external fluorescence is not detected in < 75% of the study mice. Results from the detection assay will be used as a baseline index of biomarker exposure during field-based trials.

2.5 Bait Consumption

A bait consumption study will be conducted to quantify the rate of removal of bait on the Farallon Islands. For effective mouse eradication, bait must be on the ground and available to the target animal for a minimum of ten nights to ensure consumption of a lethal quantity. A trial bait application examining the rate of bait disappearance in the environment over time, using a non-toxic replica of the preferred bait will allow for the calibration of an optimal application rate that will ensure sufficient bait availability to mice for a period of ten days.

Bait consumption rates may vary on the island in relation to changes in mouse population density throughout the year, therefore bait uptake trials should occur at the same time of year as the proposed eradication. Ideally, eradication efforts should coincide with natural seasonal mouse population declines related to a depletion of food stuffs on the island. Based on mouse trap success rates from Southeast Farallon which fluctuate widely but relatively predictably through the year, from a peak in October (71%) to a low in April (2%), the fall months would be an ideal window for the eradication to maximize natural population declines (Irwin 2006). Irwin reported capture rates in the fall can be as high as 70% and as low as 2% in the spring months. Implementing in the fall will also avoid or reduce most non-target risks, as there is very little to no seabird breeding activity on the island at that time of year.

2.5.1 Bait Uptake Calibration

An initial bait application of bait will be conducted in the first two days on island in the 0.25 ha North Landing Study Area (Figure 2) in order to calibrate what daily bait loss rates can be expected during the actual bait trial the following week. The bait application rate will be 27kg/ha during this calibration to assess whether the bait densities to be applied the following week (18kg/ha and 9kg/ha) on the Marine Terrace bait zone are sufficient to ensure exposure to all mice over the time period required for eradication. While the 27kg/ha bait density is likely more than would be used during an eradication, it will serve to determine the rate of bait loss per day, and is still within the maximum allowable registration

application rates on the registration label for brodifacoum 25D. Bait uptake will be measured in study plot transects daily using methods similar to those employed during the actual Bait Broadcast (2.5.2) described below. The calibration plot will be visually monitored for several days in an attempt to assess whether and at what rates non-target species such as gulls might forage on the bait. A small portion of the calibration plot will have a gull-exclusion device installed on it so as to assess differential uptake of bait by gulls as opposed to mice (See Section 3.1 on Non-Target Bait Uptake Assessment).

2.5.2 Bait Broadcast

After the bait loss calibration study at the North Landing study site, the non-toxic bait will be broadcast in the large trial in the Marine Terrace area of the island to the south (Figure 2). The bait will be broadcast in two applications 6 days apart in a test of the label registration guidelines. Following confirmation from the calibration trial, the first application will be at a rate of 18 kg/ha using a manual method that mimics an aerial bait broadcast. Bait will be applied to a zone ~ 5ha in area. A team of four hand-broadcasters will be spaced 10 m apart, and will walk parallel transects across the width of the study area stopping every 10 m at predetermined bait points using GPS receivers. The non-toxic bait will be uniformly applied by hand in all four directions in the 100m^2 bait area at the target rate of 18kg/ha = 180 pellets per station (~1.8 pellets/m^2).

The consumption of bait within the study areas will be measured in fixed bait uptake transects buffered at least 50 m from the edge of the baited area, or the edge of the marine terrace. The buffer zone is needed to overcome the influence of animals outside of the treatment area moving onto the bait uptake area increasing the uptake rate at the edges. Bait uptake plots will likely be 50 m long x 1 m wide. Wire flags will be haphazardly scattered in each plot at the target rate and a single bait pellet placed at the base of each flag during the application. Researchers will ensure that each plot has the number of pellets corresponding to the target application rate. Plots will be monitored every 24 hrs for up to ten days. Flags attending pellets will be left alone while flags without pellets will be removed and tallied. There will be at least eight bait uptake transects established in the baited zone (Figure 2). Some bait uptake transects may be moved or added to ensure that all habitat types are represented (*e.g.* open bare ground versus grassy thatch).

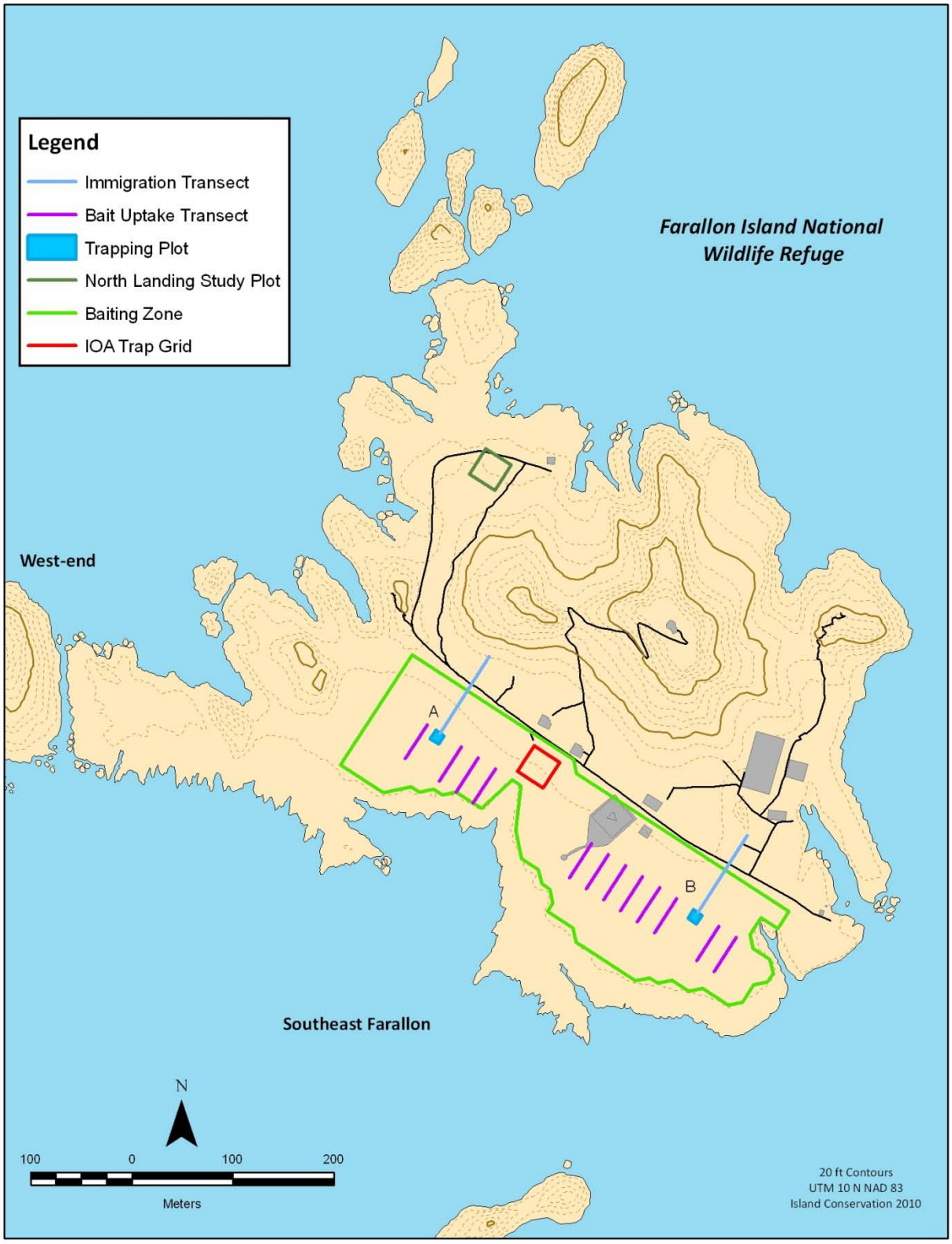

Figure 2. Trapping, Baiting Zones and Bait Uptake Areas for Southeast Farallon Biomarker Trial

The total number of bait pellets consumed from each plot will be used to extrapolate an application rate suitable for mouse eradication by aerial broadcast. The number of pellets remaining in each plot will be converted to kg/ha by multiplying the number of pellets remaining by the mean dry pellet weight, then dividing by the plot area (in ha). The consumption rate for each plot will be calculated by subtracting the remaining bait (kg/ha) from the target application rate. The mean and standard deviation of the consumption rate for all plots will be calculated and the upper 99% confidence interval for the sample mean will be used as the target application rate.

2.6 Biomarker Bait Trial

A biomarker bait study will be conducted to test the efficacy of the target application rate of Brodifacoum-25D Conservation, and to monitor exposure of non-target species to broadcasted bait. As closely as possible the methods used during the biomarker trial will mimic those for the proposed aerial eradication in order to provide a relative index of eradication outcome.

2.6.1 Biomarker Bait Broadcast

Biomarker bait 3/8" in diameter will be broadcast in two applications in the 5ha Marine Terrace bait zone (Figure 2). During the first application bait will be broadcast at the registration rate of 18kg/ha, and the second application will occur six days after the first, with bait applied at 9kg/ha, half the initial density. Bait application during the second application will follow the same standardized protocol described for the first bait application, except during the second application bait will be broadcast on transects perpendicular to those of the first application, to mimic the methods to be used during the aerial broadcasting.

2.6.2 Biomarker Bait Detection in Mice

Trapping will be conducted within and beyond the 5ha baited zone in the Marine Terrace to determine the extent to which mice are exposed to the pyranine-infused bait. Two different types of trapping will be conducted: two core trapping grids (A and B) each well within the interior of the bait zone, and two linear immigration trap grids that begin at the core but extend at least 50m beyond the baited zone (Figure 2).

2.6.2.1 Core Trapping Grid

Within the core 5ha study area mice will be trapped and screened for the presence of pyranine dye to confirm bait uptake. Two 10 x 10 trap grids will be placed near the center of the southern study site (Marine Terrace), and positioned a distance inside the plot boundary that is twice the approximate diameter of the mean mouse home range (Wanless 2009**).** Traps will be laid in pairs at 2-m intervals, totaling 200 traps per grid. Traps will be set in the evening three days after application, and trapped every night for four to five consecutive nights or for a duration as indicated in the detection assay.

Once trapped, each mouse will be screened for exposure to the pyranine dye using a hand-held ultra-violet light. External sign is generally indicated on the anus, tail, or mouth. Mice without external biomarker sign will be tail marked (using colored Sharpie markers) and released in the study area at their point of capture. For each trapping period the intensity of fluorescence will be recorded using the same rating scale previously described (see section 2.4.2). Mice that test positive for pyranine will be euthanized according to UVMA guidelines. A percentage of the exposed mice will be inspected for signs of biomarker exposure in the alimentary track. On the last day of trapping after the second application mice captured without external evidence of dye will also be euthanized according UVMA guidelines.

Euthanized mice will be buried or incinerated on the island to mimic the anticipated 87%-100% rate of underground mortality during an actual eradication. Approximately 10% of the exposed mice will be euthanized and left on the ground within 1m of the trap in the core trapping plot to allow for maximum expected secondary exposure to scavenging species, including other mice and gulls. While it is not known whether pyranine can be detected in secondarily exposed species, this trial may aid in determining this possibility. The carcasses will be inspected daily to assess the extent of scavenging, identify possible scavengers and thus help to assess secondary exposure rates. Results will be summarized to assess proportions of mice not displaying fluorescence within the core trap grid.

2.6.2.2 Immigration Trapping Transects

To investigate the effects of immigration of mice into the baited study area, an assessment transect will start at the edge of each trapping grid and run through the baited zone to at least 50 m beyond the baited area (Wanless et al. 2009). Trap stations will be placed in pairs and spaced 15m apart. Proportions of mice not displaying fluorescence on assessment lines outside vs. inside the baited study area will be compared.

2.7 DNA Sample Collection

To date, Island Conservation has been successful in all rodent eradication projects. Yet as the number, size and complexity of projects increase, so does the likelihood that a failure will occur. With the high financial, emotional, and ecological costs associated with failures, it is imperative that the causes are conclusively identified. In doing so, the procedures for future eradications can be adjusted to prevent multiple failures. Conservation biologists have recently applied molecular genetic tools to the study of population dynamics. These tools have provided information about the gene flow and the genetic structure of targeted populations. This information is crucial to the understanding of connectivity between neighboring populations, immigration events, and founder effects.

While the goal of all eradication efforts is success, if at any time after an eradication mice are again detected on an island, it is useful to be able to verify whether the source population of mice is from a new colonization event, or whether the first eradication attempt was not 100% successful. In order to establish this, a sample size of DNA of approximately 50 samples per island in the South Farallon Island group will need to be collected and stored prior to the eradication. DNA collection methods and protocols used will be those described and used in previous IC rodent eradications (Ross 2009). During the three week biomarker trial study period, tail tissue from mice euthanized during trapping will be collected and stored in labeled vials with Lysis buffer solution and stored for future use, if needed. Additional samples can be collected from West End and all other potentially occupied surrounding islets during this study and at any time prior to eradication.

3. Biomarker Detection and Bait Uptake by Non-target Species

Compared with most circumstances on the mainland, island ecosystems are generally better candidates for aerial broadcast of rodenticide for rodent eradication because there are fewer other animal species present to consume the bait and potentially succumb to its toxic effects. On the South Farallon Islands, a bait broadcast would be timed for the winter months when there will be comparatively few animals present, further minimizing “non-target” exposure to rodenticide. However, during the months of November and December, when the bait broadcast is currently proposed, gulls may be present in variable numbers on any given day. Endemic arboreal salamanders are also present on the island and may be active during these times of year, depending on the rainfall. While none of these taxa are believed to be at risk of significant, long-term negative population impacts from an aerial rodenticide broadcast, the scale of their

potential exposure to the bait on the South Farallones is not easily predictable prior to trial studies to assist in this assessing the rate of possible exposure.

In conjunction with biomarker bait trials to assess the efficacy of mouse eradication, two potential non-target species (gulls and salamanders) will be examined to assist in the development of an index of the potential risk of exposure to rodenticide during the proposed eradication. A bait application trial using non-toxic biomarker-infused bait will allow researchers to quantify exposure rates in gulls, which may consume bait pellets if they encounter them. Due to their insectivorous diet, salamanders are not likely to be exposed directly to rodenticide, but their native (and possible endemic) status to the South Farallons warrants taking additional measures to examine all potential exposure pathways to the rodenticide. The major focus of this non-target trial study will be on the gulls, and to a lesser extent on the salamanders and invertebrates.

3.1 Western Gull residents and Migratory Gulls

To assess the risk associated with bait application during the proposed eradication operation, resident Western gulls and migratory gull species such as California gulls and Herring gulls will be evaluated for exposure to the biomarker during the trial. During the proposed eradication period of November to December, variable numbers of resident Western gulls return daily to roost on the island on or possibly near their territories which are scattered widely across the islands. Migratory gulls also roost on the island in large numbers, but tend to congregate in the intertidal areas with some spillover onto upland areas. Whether migratory gulls utilize the intertidal and upland areas for foraging purposes is unknown, but it is likely variable. Gulls begin setting up on territories in late November in anticipation of breeding around December, with egg-laying usually beginning in April (Ainley, 1990).

3.1.1 Non-breeding Season Gull Counts

Counts are being conducted daily by PRBO from Oct. 1 to Dec. 31, 2010 to assist in assessing the number of gulls present and roosting on the island during the fall non-breeding season. These consist of evening roost counts along the perimeter of most of SEFI and the eastern portions of West End. On a weekly basis, additional surveys are being conducted from the lighthouse (and additional areas) to help calibrate these partial roost counts to develop an approximate island-wide census for gulls during the fall period.

3.1.2 Gull Uptake Exclusion Plots

In order to separate the uptake rate of bait pellets by gulls as opposed to by mice, up to six gull exclusion plots will be established within the baited zone in areas near or in where gulls are known to roost. Wood and chicken wire cages ~2.4 meters on a side and 2 feet tall (8 feet square) will be set out to allow mice to enter at ground level, but should preclude gulls and other large birds from gaining access to the pellets. If there is a differential in bait uptake in these exclusion zones, it will be possible to extrapolate what the target and non-target uptake rates might be by measuring bait uptake in side and just outside of the exclosures.

The bait calibration study at the North Landing Study site will serve to provide a test site to determine whether and at what rates gulls might consume the bait pellets. A ~5m x 5m area within the 50m x 50m baited zone at this location will house a gull exclusion device to determine the uptake differential of bait by gulls as opposed to mice**.**

Up to six exclusion devices will likely be placed strategically throughout the Marine Terrace bait zone close to gull roost sites and/or where the Index of Abundance trap grid is located (Figure 2). This general area was selected so as to be a maximum distance from the two core bait trapping grids, so as not to unduly influence the trapping results. The exclusion devices might be arranged in separate locations in this area or could be set up adjacently to provide for one large gull exclusion area approximately 5m x 5m square. Uptake will be measured inside the six exclusion zones, as well as a paired area of equal size immediately adjacent to the exclusion zones. Bait will be marked with wire flags to aid in daily pellet counts.

3.1.3 Gull Foraging Observations in the Bait Zones

After the broadcast of biomarker bait in the calibration plot at North Landing an observer will be stationed for most of the day to observe the baited areas from a distance using binoculars or spotting scopes to record the number of gulls (and any other non-target species) foraging on bait. Foraging rates of individual gulls consuming pellets will be recorded and the number of potentially different gulls observed consuming the non-toxic bait pellets on the plot will be recorded. Following a potential ingestion event(s) a close-up inspection might be conducted to attempt to confirm the direct pellet consumption event and to more accurately assess uptake rates and amounts by gulls. Because of the size of the main 5ha bait zone on the Marine Terrace, and limited staff time available,

only portions of the day will be possible to devote to directly observing the baited zone to detect possible foraging events by gulls or other non-target species. Up to 2 hours per person each day might be possible to devote to such observations, but exact times and methods will need to be flexible in order to carry out all of the essential aspects of the trial.

3.1.4 Secondary Gull Exposure due to Predation/Scavenging of Mice

To assess possible secondary exposure, to mimic an actual eradication event, 10% of the mice trapped in the core sampling grid that were exposed to the bait (via the pyranine dye assay) will be euthanized and set out on the trap grid in locations marked for subsequent daily monitoring. This may mimic the percentage 0-13% of mice which may expire above ground in an actual eradication using the bait formula with brodifacoum. The quick disappearance of a mouse carcass will be categorized as a possible gull scavenging event, unless other another fate can be determined, such as scavenging by other mice. Gulls will also be observed in the trap zones daily in order to detect and record to any ingestion of pyranine-exposed mouse carcasses by gulls from a distance. It is unknown whether the water-soluble pyranine dye will be detectable in gulls when the ingestion is a secondary pathway.

Note: **To carry out the gull studies below, the advice and assistance of experienced PRBO researchers on the Farallon Islands will be required, particularly those with knowledge relating to gull roosting, capture and foraging behavior of gulls. Permitted PRBO staff will be needed to assist in attempting to capture, mark and color-band up to 100 gulls during November 2010 and to conduct recounts of banded gulls to assist in determining the number of gulls present, and the rate of turnover of individual gulls from day to day.**

3.1.5 *Gull Capture and Biomarker Inspection*

Attempts will be made to capture of a subsample of birds from each study area after bait broadcast within or immediately adjacent to study areas. Beginning 3 days after each bait application, gulls will be trapped either individually (spotlight or snares at their roost sites) or en masse (box trap) for five consecutive days. Birds will be baited with fish or other likely bait. Gulls caught will be examined externally around

the bill area and cloaca for evidence of fluorescence using a UV light. The age class and presence or absence of fluorescence will be recorded for each captured bird examined. Following examination, birds will be marked with a temporary dye (picric acid) on the chest or head and banded with a red color band on their right leg and released at their capture location. This marking will be easily visible when leg bands may not be, and will assist in avoiding double-counting. The proportion of marked gulls returning to roost can then be better determined during the evening gull roost surveys conducted daily by PRBO biologists. The number and percentage of gulls showing external exposure to the pyranine marker will be recorded.

3.1.6 Gull Exposure Counts by Remote Observation

Given the potential challenge of capturing non-breeding gulls and the larger spatial movements of gulls outside the study area, distant visual observations of roosting gulls will be made to examine for fluorescence. Visual observation of roosting gulls from a distance will be attempted using an ultra-violet spotlight within and surrounding the study areas. A UV spotlight will be used to illuminate bird fluorescence visible around the mouth and anus area at each known roosting site on the island. Observers will be positioned ~ 50 m, or as close as possible without flushing birds, and the numbers of birds by age class displaying or not displaying fluorescence tallied. Beginning 3 days after bait application counts will be made during the evening for five consecutive days. The proportion of gulls at each roosting site displaying fluorescence will be recorded. During these surveys anecdotal observations of other bird species that could have also been exposed to bait will also be recorded.

3.1.7 Gull Fecal Plot Counts

Evaluation of gull feces on the island will be randomly assessed during the first few nights on the island at night using a UV light to determine if there are any naturally occurring fluorescent materials (such as squid) in the gulls' diet. Prior to baiting specific gull fecal plots will be selected and marked in or near the bait zone. Fixed plots (approximately 5m x 5 m) will be established targeting known gull roosting sites. Prior to bait application fecal deposits within each plot will be cleared of feces. Following bait application, plots will be repeatedly visited beginning 3 days after each bait application for five consecutive days. New fecal deposits within the plot will be examined for evidence of fluorescence using a UV light. At each visit the number of new deposits with or without fluorescence will be tallied, and all new deposits cleared from the plot using saltwater and brush, to reduce the likelihood of pyranine leeching during precipitation, potentially resulting in subsequent false-positive detections.

Information gathered in the above studies will be summarized as proportion of roosting gulls that detected positive. The data will be used one of two ways: a simplistic model quantifying risk exposure to gulls on the Farallon Islands, based on a proportion of the roosting population exposed to biomarker. This and all other relevant gull data collected during the trial will be provided for a more in depth risk assessment that is being conducted by experienced risk assessment modelers and analysts.

3.2 Salamanders

No native reptiles or mammals inhabit the Farallon Islands, but arboreal salamanders (*Aneides lugubris*) are found across the islands. The species is endemic to mainland California and Baja California where it is distributed primarily along the coast, with populations on some offshore islands and in the Sierra Nevada foothills.

Although not threatened, salamanders will also be evaluated for exposure during the biomarker trial. Salamanders are not expected to consume bait pellets directly, but their status as an endemic subspecies warrants further investigation. One month prior to bait application, 50 temporary well seasoned cover-board shelters (2" x 2" x 12") preferred by salamanders were installed within the study area (Figure 3). Beginning 3 days after each bait application and continuing for 5 consecutive days the shelters will be checked and any salamanders captured will be examined for fluorescence using a UV light. The presence/absence of biomarker fluorescence around the mouth or vent will be recorded for each captured salamander. Additional temporary shelters will be examined for fecal material or invertebrates showing fluorescence. To avoid double-counting, salamanders will be photographed to determine number of individuals, per methods used currently on island by PRBO.

Information gathered in the above studies will be summarized as proportion of salamanders that detected positive. The data will be used one of two ways: a simplistic model quantifying risk exposure to salamanders on the Farallon Islands, based on a proportion of the population exposed to biomarker.
It should be noted that results from this study may yield a false positive, as salamanders might be exposed to the dye owing to its solubility in water and the salamanders' preference for damp habitats (for example bait deteriorating in rainwater puddles and releasing the dye).

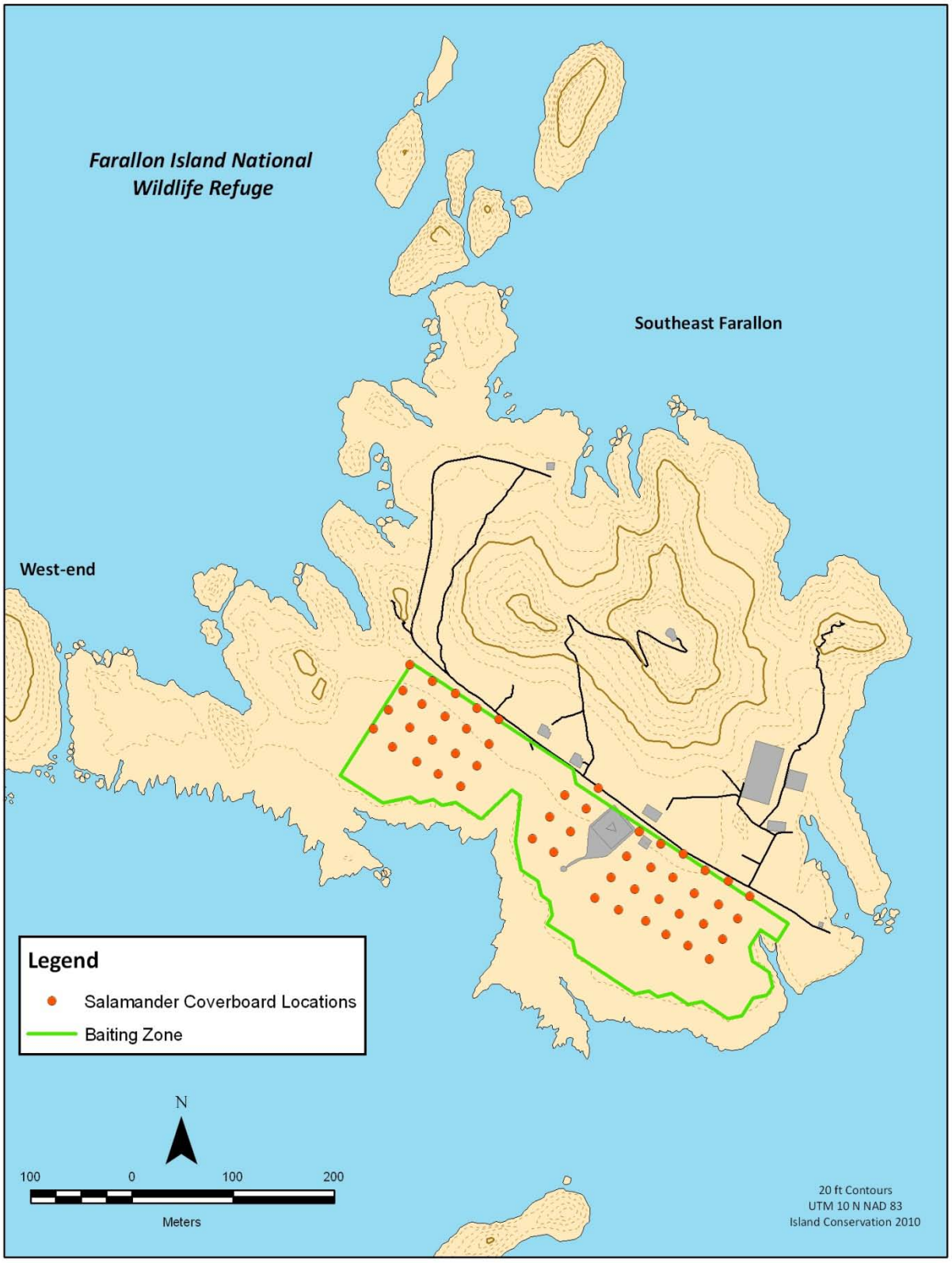

Figure 3. Salamander Cover Board Locations within Biomarker Baiting Zone

3.3 Invertebrates

Carabid beetles and other commonly occurring terrestrial invertebrates will be assessed randomly throughout the baited area during the week following the bait applications during mouse trap baiting and checking activities to assess for exposure to the pyranine biomarker. Percentage of exposed and non-exposed individuals will be calculated.

3.4 Burrowing Owls

While the continued presence of burrowing owls pose a predation threat to many native breeding seabird species on the island, attempts to limit the exposure of the bait to owls and other non-target species during the eradication may be pursued. Current plan are to capture as many of the vagrant burrowing owls present on island just prior to an eradication and to repatriate them back to the mainland so as to avoid exposure. However, since the burrowing owls will be present during the biomarker trial, and since some will even be equipped with radio-transmitters, they will be observed when possible at selected periods of the day to assess their foraging behaviors in the baited zones. If possible, a few of these owls will also be approached at night with a UV spotlight in order to detect if any biomarker is visible around the bill or cloaecal areas, just in case secondary exposure to pyranine is detectable. Additional UV assessments will be conducted of burrowing owl pellets found on island during pre-baiting and post-baiting periods to determine if insects or other prey items exposed to the bait show up in the casts.

4. Climate Data

Standard climate data will be collected by biologists on island during the bait application trials to account for some of the variables that might influence the results of any of the studies. This will include daily rainfall, minimum and maximum ambient temperatures, wind-speed and direction and approximate cloud cover. PRBO records contain accurate weather data extending back to 1968 for historical purposes, and for use in anticipating the average numbers of mice and gulls on the island in any given day or month of the year.

5. Field Schedule & Resource Needs

5.1 Field schedule

A proposed schedule of field activities is shown in Table 1. The initiation date is set for November 1st, and the termination date of the trial will likely be on or around November 23rd. While a total maximum time of 23 days will be required to conduct the field trials as described, the duration of the field trial could be reduced, depending on the pyranine detection period in mice as determined in the lab assay trial. Field trial time could also be extended, depending on weather and other delays. The exact calendar dates of the trial are not fixed, and will be subject to staff-time available, logistical considerations, weather and unanticipated trial assessment results.

5.2 Staffing

To complete the trials as described a core group of 4 Island Conservation staff will be required. This group includes Dan Grout (Project Manager), Jake Bonham, Maddy Pott and Erik Oberg. Additional part-time support of 2-3 PRBO or U.S. Fish and Wildlife Service biologists will be required to assist and advise on many of the gull-related aspects of the study, and to a lesser extent for the burrowing owl, salamander and invertebrate studies.

5.3 Field Staff Accommodation

Accommodation for the four Island Conservation staff during the trial has been provided by the USFWS in the former Coast Guard house. Island Conservation will coordinate appropriately on a daily basis with PRBO and USFWS staff on and off-island regarding specific field logistics.

5.4 Field Resource Needs

Island Conservation researchers will need to coordinate with FWS/PRBO staff regarding logistics, timing, and transport of field staff and equipment to the island. During field trials IC researchers will attempt to remain as self-sufficient as possible so as not to disrupt ongoing PRBO research, but IC staff might at times request and require access to shared laboratory and office space to conduct some of the studies and analysis mentioned above. Researchers will require access to basic utilities on the island, including

power for charging camera and laptop batteries, restrooms, and water, while maintaining awareness of and compliance with standard operating procedures for water and power.

Prior to and during the transit to SEFI on Nov. 1, the USFWS Refuge Manager will provide IC staff with a briefing on the USFWS/PRBO safety and communication plan procedures. IC staff will communicate with PRBO biologists using handheld radios when necessary, and will undergo a safety briefing and coordinate activities with PRBO staff on island on a daily basis.

A resupply of perishable fresh food and water and other supplies might be necessary for the four IC staff once or twice during the three week field survey, most likely during the period between November 10th - 17th. It would be appreciated if PRBO and/or USFWS staff could provide IC staff with advance notice as to the possible dates and times of possible resupply vessel options, including the Farallon Patrol, USFWS charter vessel or the daily shark research boat.

Transit off island is currently scheduled to occur by helicopter on Nov. 22, weather permitting. Any remaining equipment that is not needed by IC, PRBO or FWS for work in the immediate future that does not transit transit off island on the helicopter will be transported off-island in the subsequent days/week by returning empty boats as space and weather permit.

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Table 1. Timeline of Activities for Farallon Biomarker Bait Trial (Attached Excel version is easier to read than Table below)

Category	Activity (Oct.12 Draft)	Person-Hrs	Time	NOV. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25 -Holiday
General	Transit island, orient, unpack	3, 1, 2	7am-noon	x																								
General	Baseline UV surveys (mice, gulls, gull plo	3p/hr	7-9pm	x																								
Bioassay trial	Set and check bioassay traps	1p/hr		x	x																							
Bioassay trial	Establish bioassay: feed pellets to mice	2p/hr			x																							
Bioassay trial	UV Inspect bioassay subjects for marker	1p/hr				x	x	x	x	x	x	x	x	x														
Bait Loss Calibration-27kg	Establish bait loss plot @ North Landing	?	1-3pm?	x																								
Bait Loss Calibration	Assemble gull exlosures & broadcast bait	4-5p/hrs	1-5pm?	?	x																							
Bait Loss Calibration	Observe gulls foraging in calibration plot	2hrs? + ?	eve. & am?		x	x	x	x	?	?																		
Bait Loss Calibration	Measure bait loss in NLplot & exclosures	2 p/hrs?	afternoon		?	x	x	x	?	?																		
Baiting field trials	Establish 2 core trap grids-set traps out	8p/hr	afternoon		x																							
Baiting field trials	Establish Broadcast points-flag every 5?	30 p/hr	anytime		x																							
Baiting field trials	Establish ends-uptake transects-flag	4 p/hr	anytime		x																							
Baiting field trials	Establish immigration transects-set traps	1-2 p/hr	afternoon		x																							
Mouse IOA	Set/Open IOA traps (Grid and Irwin)	2-3 p/hr	3-6 pm	x	x	x	x	x													?	?	?					
Mouse IOA	Check IOA traps & mark mice	2-4 p/hr	7am-10am		1.1	1.2	1.3	1.4	1.5													?	?	?				
Mouse IOA	Remove IOA traps (Irwin on 3 & Grid -5)	2 p/hr	morning				x		x															?				
Baiting field trials	Broadcast biomarker plot @ 18 kg/ha	12p/hr?	noon-5pm						x																			
Baiting field trials	Measure bait uptake 18 kg/ha	4p/hr	morning							1.1	1.2	1.3	1.4	1.5														
Baiting field trials	Open & Bait sherman traps in core areas	4-6 p/hr	3-5pm								x	x	x	x														
Baiting field trials	Inspect trapped core mice for biomarker	8 p/hr	6am-8am									1.1	1.2	1.3	1.4													
Baiting field trials	Inspect mice in mmigration traps	1 p/hr	6am-7am									1.1	1.2	1.3	1.4													
Baiting field trials	Inspect alimentary tracts & set out mice	2-3 p/hr	10-12am									x	x	x	x													
Baiting field trials	Assess carcass uptake	2 p/hrs?	afternoon										x	x	x	x	x	x	x	x	x	x	x	x	x			
Baiting field trials	Close Sherman traps	1 p/hr	6am-8am												x													
Baiting field trials	Broadcast biomarker plot @ 9 kg/ha	12 p/hr	noon-5pm												x													
Baiting field trials	Measure bait uptake	4 p/hr	afternoon													2.1	2.2	2.3	2.4	2.5								
Baiting field trials	Open & Bait sherman traps	4-6 p/hr	3-5pm														x	x	x	x								
Baiting field trials	Inspect trapped core mice for biomarker	8 p/hr	6am-8am															2.1	2.2	2.3	2.4	2.5	?					
Baiting field trials	Inspect mice in immigration traps	1p/hr	6am-7am															2.1	2.2	2.3	2.4	2.5	?					
Baiting field trials	Inspect alimentary tracts & set out mice	2-3 p/hr	10-12am															x	x	x	x	x						
Baiting field trials	Assess carcass uptake	2p/hr	afternoon																x	x	x	x	x	x	x			
Baiting field trials	Pick up and clean Sherman traps	8p/hr	morning																			x						
Gull uptake surveys	Establish gull uptake plots-Marine Terrac	4p/hr	anytime		x																							
Gull uptake surveys	Flag and Hand-set/gull uptake plots	4p/hr	anytime						x						x													
Gull uptake surveys	Measure bait uptake - Marine Terrace	1p/hr	anytime							1.1	1.2	1.3	1.4	1.5	?	1.1	1.2	1.3	1.4	1.5								
Gull biomarker surveys	Gull counts and feeding behavior/rates	???	?		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Gull biomarker surveys	Pre-bait (?) and Capture gulls	????	Check with PRBO									x	x	x	x	x	x	x	x	x	x	x						
Gull biomarker surveys	Establish gull fecal plots	1-2 p/hr	anytime			x																						
Gull biomarker surveys	Wash gull fecal plots	1p/hr	am								x	x	x	x	x	x	x	x	x	x	x	x	x	x				
Gull biomarker surveys	Examine fecal plots	1-2 p/hr	am?/pm?									x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Gull biomarker surveys	UV Spotlight gull observations	1-2 p/hr	night	x	?							x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Salamander biomarker su	Mark(#) & Confirm coverboard locations	1 p/hr	anytime		x																							
Salamander biomarker su	Inspect salamanders for biomarker	1-2 p/hr	mid-day?					x			?	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Invert biomarker surveys	During trap sets/checks & as time allows	1p/hr	am/pn					x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
DNA collection	Collect DNA from Sherman trapped mice	1-2 p/hr	morning						x			x	x	x	x													
DNA collection	Set snap-traps; collect DNA in bldgs	1 p/hr	am/pm	x																								
DNA collection	Collect DNA from other strata(West end)	3hrs	anytime			x																						
General	Demobilize and stow gear		anytime																				x	x				
General	Transit off island by helicopter		1pm?																						x			Holiday
(Sunrise~7:30, SSet: 6pm;	*Nov. 7 onward: SR: ~ 6:30am, SS: ~5pm)*																											