Review of the Farallon Feasibility & Non-target Risk Assessment Report

December 6, 2013

*Completed for:*

U.S. Fish and Wildlife Service

San Francisco Bay National Wildlife Refuge Complex

*Completed by:*



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Review Summary

Overall, the trials report was thorough and addressed several important issues that should be considered during the planning and preparation for the mouse eradication on the Farallon Islands.

It is important to stress the need to understand and acknowledge the degree of risk resulting from the presence of gulls on the islands during the eradication operation. The risk includes the potential for significant gull mortality resulting from consumption of the rodenticide bait, but also a risk to the success of the eradication operation resulting from bait competition between the gulls and mice (creation of bait gaps). Additional research into the viability of various hazing techniques is highly recommended (this was mentioned in the report). Furthermore, I anticipate that bait uptake experiments have been planned to help determine the optimal application rates, but there is no mention of them in the report.

**1) Were the methods used appropriate?**

The methods used were appropriate in order to provide preliminary data that can help the eradication planning team to better understand the challenges that a mouse eradication on the Farallon Islands presents.

**2) Were the analyses used appropriate?**

Yes, although the bait availability trial could have been used to help determine the bait application rate using bait uptake plots. These trials may have already been completed or are being planned.

**3) Were interpretations of the results appropriate?**

For the most part, yes. Please refer to my comments within the report for additional information.

**4) Suggestions for improvement.**

I included comments directly in the report concerning this. There is a need for additional research into the bait application rates based on bait uptake trials, in addition to an experiment to critically assess the utility of various gull hazing techniques (this was mentioned in the report).

**Farallon Islands Restoration Project**

**A Report on Trials undertaken to inform   
Project Feasibility and Non-Target Risk Assessments**



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# **EXECUTIVE SUMMARY**

A field trial completed in November 2010 was successful in addressing several objectives identified as important in planning for a proposed eradication of invasive house mice on the South Farallon Islands of the Farallon National Wildlife Refuge. The results from the trial will inform the development of eradication alternatives as well as possible non-target mitigation measures to be considered during project planning.

Key findings of the trial were as follows:

* Mice were exceptionally abundant on the South Farallon Islands in November 2010, with over 93% trapping success and more than 250 uniquely marked individual mice captured within a 0.25ha study site. Mark-recapture data indicated mouse densities of up to 1297 mice per hectare, representing one ofthehighest recorded population densities for anywhere in the world.
* Mice were distributed across the island including West End but variation in density from site to site was high. Many mice were active during the day during the fall months on the South Farallon Islands.
* Although mice in reproductive condition have been trapped year round on the South Farallon Islands, very few mice were found to be reproductively active in November. Reduced breeding activity and apparent food scarcity at this time of year marks this season as the best in which to undertake a mouse eradication.
* Mice exhibited no sign of any Vkorc1 alleles associated with anticoagulant resistance, confirming there is no known genetic barrier to successful eradication if anticoagulants were to be used.
* A 1g cereal bait pellet containing the fluorescent dye pyranine was readily accepted and appears to be highly palatable to Farallon mice.
* Applying rodent bait at 18kg/ha provided four days of bait availability after an initial application. Only one to two days of availability was achieved following a subsequent application at 18kg/ha in one area and 9kg/ha in another. The period over which bait will be available is expected to be longer during an operation as mouse numbers will be reduced after the first application of bait and if consumption of bait by gulls can be minimized. Consequently, EPA label rates of 18kg/ha and 9kg/ha specified for Brodifacoum-25D Conservation are considered sufficient to ensure that all mice have time to consume sufficient bait to ingest a lethal dose for an eradication operation utilizing a second-generation anticoagulant as the rodenticide.
* Following the application of rodent bait 18 kg/ha and 9kg/ha more than 96% of trapped mice showed evidence of exposure to bait. For similar reasons as those stated above, EPA label rates of 18kg/ha and 9kg/ha are considered sufficient for an eradication operation to ensure all mice are exposed to bait.
* Western gulls were observed consuming rodent bait and it is concluded that individual western gulls present on the islands during a mouse eradication would be at risk of primary and secondary poisoning. The implementation of a hazing program is recommended to prevent western gulls from consuming bait pellets and inhibit learnt behavior.
* Consumption of rodent bait by gulls could reduce the amount of bait available to mice and hazing of gulls is recommended to maximize the likelihood of mouse eradication success.
* No exposure to pyranine (a fluorescent dye) was observed in two burrowing owls inspected during the trial now any of the fecal pellets found. However, individual burrowing owls present on the island are still considered to be at risk because they are expected to consume poisoned mice.
* The hand-broadcast of non-toxic bait pellets containing a fluorescent dye in salamander habitat on the island found no evidence of salamander or invertebrate exposure. Camel crickets exposed in the same way did consume trace amounts of the cereal grain pellets. However, camel crickets, because of their physiology, are not at risk from anticoagulants such as diphacinone and brodifacoum.
* Two bait station designs tested were readily used by mice and successfully excluded gulls.

# **INTRODUCTION**

The South Farallon Islands, comprised of Southeast Farallon Island (SEFI) and West End Island (WEI), provide important habitat for seabirds and pinnipeds, and support some of the world’s largest seabird populations including Ashy Storm-Petrel (Oceanodroma homochroa), Brandt’s Cormorant (Phalacrocorax penicillatus) and Western Gull (Larus occidentals ([Ainley and Boekelheide 1990](#_ENREF_1), [Warzybok and R. 2011](#_ENREF_24)). House mice (Mus musculus), introduced to the South Farallon Islands sometime during the 19th century, indirectly and possibly directly affect burrow nesting seabird populations and are expected to be impacting other native and endemic species.

The impacts of House mice on species and ecosystems are described in Mackay ([2011](#_ENREF_15)). As observed on other islands around the world, introduced house mice pose a significant threat to seabird populations ([Ainley and Boekelheide 1990](#_ENREF_1), [Sydeman et al. 1998](#_ENREF_22), [Cuthbert and Hilton 2004](#_ENREF_6)). On the South Farallon Islands, mice also provide a food source that supports an overwintering population of migratory burrowing owls, which in spring switch to Ashy Storm-Petrels (Oceanodroma homochroa) as prey. Ashy Storm-Petrels are a rare species whose largest breeding colony occurs on the South Farallon Islands ([Carter et al. 2008](#_ENREF_4)). Other recorded impacts of mice include predation or competition with many native and endemic reptile and invertebrate species ([Newman 1994](#_ENREF_17), [Ruscoe 2001](#_ENREF_20)).

To eliminate these impacts and allow species and ecosystem recovery, the USFWS is currently assessing the potential for removing mice from the Refuge. A series of trials has been completed to inform planning for a possible eradication attempt. This report documents the findings of recent trials that aimed to assess the efficacy of eradication techniques, quantify potential risks to non-target wildlife and evaluate a potential mitigation measure to reduce risk to non-target species.

Although a wider suite of methods is under consideration, trials focused on the use of rodent bait containing an anticoagulant rodenticide. The application of anticoagulant rodenticides is the only method that has been used successfully to remove mice from islands ([Keitt et al. 2011](#_ENREF_13), [Mackay et al. 2011](#_ENREF_14)). Early analysis of options for the removal of house mice identified gulls as a potential non-target species at risk from a mouse eradication ([Howald et al. 2003](#_ENREF_11)). Although widely distributed along the western US seaboard, the South Farallon Islands are home to the world’s largest colony of western gulls (Ainley and Boekelheide 1990). Consumption of rodent bait poses not only a risk to gulls but also to the operation, as gulls could consume sufficient bait to create gaps in bait coverage. Successful eradication of mice requires all individuals within the mouse population to be exposed to the technique ([Bomford and O’Brien 1995](#_ENREF_3)).

Native reptiles and terrestrial mammals are absent from the Farallon Islands, but an amphibian, the Arboreal salamander (*Aneides lugubris farallonensis*) occurs on Southeast Farallon Island. 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. The Farallon subspecies is not considered threatened but is only found on the South Farallon Islands. Farallon salamanders are primarily insectivorous, are not considered at risk from the application of rodent bait and are expected to benefit as a result of mouse eradication ([Newman 1994](#_ENREF_17), [Baber et al. 2007](#_ENREF_2)). However, their endemic status warrants additional analysis and risk to salamanders was assessed as part of our trials.

The endemic Farallon camel cricket (*Farallonophilus cavernicolus*) is not considered to be at risk and evidence ([e.g. Green et al. 2011](#_ENREF_9)) suggests that cricket abundance will increase on the islands once House mice are removed. A pilot census was undertaken in accessible caves on Southeast Farallon to inform the development of baseline surveys to monitor relative cricket abundance before and after mouse eradication.

In the event that mice are detected on the Farallon Islands after the proposed eradication, knowing the provenance of individuals is important to verify whether the eradication failed or the island biosecurity system was breached. For this reason, samples of mouse DNA were collected from SEFI and WEI for long-term storage and future analysis. Genetic analysis was also undertaken to confirm the subspecies of House mouse present, their geographic origin, and to determine if mice on the islands are resistant to anticoagulants.

# **OBJECTIVES**

* Assess mouse abundance by using mark-recapture techniques and establish protocols for tracking seasonal changes in mouse abundance on SEFI.
* Determine the reproductive status of mice during the fall.
* Determine the persistence of the fluorescent dye pyranine in mice.
* Evaluate the palatability of proposed bait to mice and their preference for this food over natural food sources.
* Apply a non-toxic bait product to a portion of SEFI in order to assess the availability of bait pellets over time and the proportion of the mouse population exposed to bait pellets.
* Collect and archive samples of DNA from island mice.
* Confirm if South Farallon Islands mice are resistant to anticoagulant rodenticides.
* Assess the risk of primary or secondary rodenticide exposure to western gulls, burrowing owls and salamanders using a non-toxic bait applied at the target application rate.
* Determine if camel crickets will eat rodent bait.
* Identify a potential method for monitoring the change in abundance of camel crickets over time.
* Determine acceptability of two bait station designs to mice.
* Confirm the effectiveness of two bait station designs to isolate gulls from bait exposure.
* Map and characterize caves to inform operational planning for a future mouse eradication attempt.

# **METHODS**

# **Mouse Abundance**

*Index of Abundance*

Prior to broadcasting rodent bait, a 45m x 45m grid of 100 traps spaced at 5m intervals was set and checked for five consecutive nights within the intended baiting zone in order to develop an Index of Abundance for mice (Fig. 1).

*Monthly mouse trapping*

Thirty three permanent mouse trapping locations were established on SEFI for conducting monthly mouse trapping as a means of establishing a monthly index of activity throughout the year. In addition to the 28 sites previously used in USFWS mouse trapping studies conducted from 2001-2004 ([Irwin 2006](#_ENREF_12)), five new locations were established in the Lighthouse Hill area to obtain a more representative sample from this habitat type. Sites were marked with white PVC, aluminum tags, and had GPS coordinates recorded (Fig. 1).

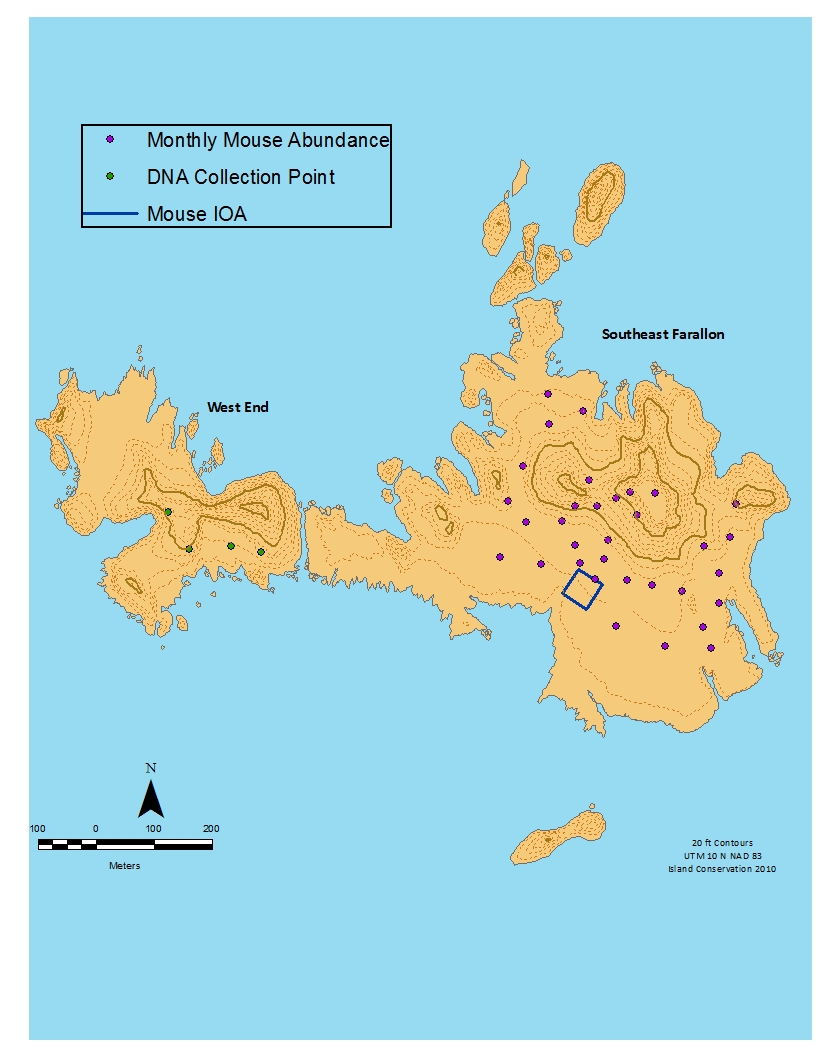


Fig. 1. Location of the Index of Abundance trapping grid and monthly mouse trapping locations.

# **Mouse Reproductive Status**

All mice trapped during our trials were assessed for reproductive activity, including descended testes in males and perforate vaginas and enlarged mammae in females.

# **Biomarker Persistence in Mice**

To guide our interpretation of the mouse exposure field study described below, a study of captive Farallons mice was used to determine how long pyranine persists in the gastrointestinal tract after consumption. Pyranine fluoresces green when exposed to ultraviolet light (UV). Twelve mice were fed a non-toxic form of Brodifacoum-25D Conservation (Bell Laboratories, Inc. Madison, WI, EPA Reg. No. 56228-37) infused with 0.2% pyranine during a six-day no choice trial undertaken on the island. Two mice were also kept as a control.

The twelve mice were divided into three different exposure groups with four mice in each group. Two adult males and two adult females in good condition were randomly placed in each group. On the first day of the study, mice in Group 1 were fed an amount of non-toxic bait equivalent to half the amount of Brodifacoum-25D Conservation required for ingestion of a LD50 (approximately 0.5 g). Mice in Group 2 were fed an amount equivalent to the LD50 (approximately 1 g) and Group 3 was fed twice the LD50 amount (approximately 2 g). Quantities were based on estimates that a mouse must eat 1-2.6% of its body weight of 20ppm brodifacoum bait to achieve acute oral toxicity ([Fisher 2005](#_ENREF_8)). Mice in the exposure group were fed non-toxic pellets without pyranine on the second, third, and fourth days of the trial. All mice were individually housed and provided with *ad libitum* water.

All mice were checked daily for four days for the presence of fluorescence under UV light at both the mouth and the anus.

# **Bait Palatability and Preference**

A two-choice food preference trial was conducted to determine consumption rates and food preferences. The tests were conducted in a laboratory setting on-island and continued for eight days, with each mouse housed individually. Ten adult mice were given a choice between non-toxic bait pellets with pyranine and locally sourced natural food alternatives included coleopteran larvae and fresh local vegetation (endemic *Lasthenia maritima* and invasive *Hordeum murinum leporinum*). The natural foods used in the trial were selected based on a description of Farallon mouse diet by Hagen ([2003](#_ENREF_10)). Each mouse was supplied daily with 2.8g of bait pellets and 2.06g of the naturally occurring food items, totaling 4.86g of food per day. Every day, the amount of each food type (natural food or bait pellet) consumed by individual mice during the previous 24 hours was determined based on the amount of food remaining in the cage.

# **Rodent Bait Availability**

In order to assess the bait application rates required to ensure all mice have access to a lethal dose of bait during an eradication operation a bait availability trail was undertaken on SEFI. To provide an indicator of a starting application rate to use in the trial non-toxic bait was initially hand broadcast at 36kg/ha over a 0.25 ha plot at North Landing (Fig. 2). Based on observations of bait disappearance from this area, a larger 6.2 ha plot was split into two: Area A (western half) measuring 3 ha and area B (eastern half) measuring 3.2 ha. Non-toxic rodent bait was initially hand broadcast at a density of 18 kg/ha in both areas. Five days later, bait was hand broadcast at 18 kg/ha in Area A and 9kg/ha in Area B.

Immediately after bait had been hand broadcast, 10 bait availability monitoring transects (six in Area A and four in Area B) of 1 m x 50 m were calibrated so they contained the number of pellets representative of the bait application rate used in that area. Transects were then checked daily to determine the availability of bait pellets over time (Fig. 2). In an attempt to assess how the availability of pellets was affected in the absence of gull consumption, four exclusion cages (two in each area) were established (Fig. 2). The 2.4m x 2.4m exclusion cages were made of wood and chicken wire and allowed mice to enter and feed on bait pellets, but prevented gulls from accessing bait. Bait pellets within exclusion cages were counted on a daily basis.

# **Mouse Biomarker Exposure Rates**

An indication of efficacy can be gauged by measuring exposure rates to non-toxic bait infused with pyranine. A core trapping grid was established in both Area A and B (Fig. 2). Two traps were placed at each point of a 2m x 2m grid across an area of 18m x 18m. On the second day following each bait application, trapping was initiated and continued for a total of two nights. Traps were checked daily and captured mice were assessed for exposure to pyranine. All mice testing positive for exposure were removed from the population each day.

Immigration transect trapping was conducted concurrent with core grid trapping in both Areas A and B. Each transect extended from the edge of the core trapping plot to at least 90 meters beyond the edge of the baited area (Fig. 2). Two traps were placed at 10m intervals along the transect. Traps were opened concurrently with core trapping grid traps and were checked in an identical fashion.

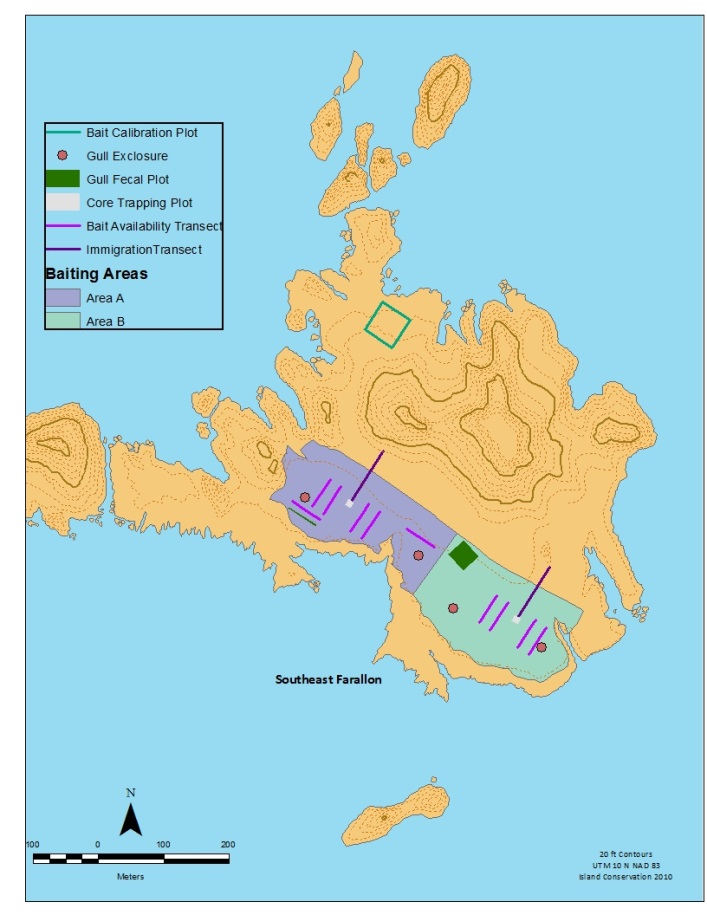
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Fig. 2. A map of baited areas, availability transects, immigration transects, core trapping grids, gull fecal plots, and gull exclusion cages

# **Mouse DNA Sampling and Genetic Analysis**

In the event that mice are detected on the islands subsequent to an eradication attempt, archived DNA samples will allow a determination of whether the operation failed or mice were reintroduced. Tail tissue samples were collected from a number of locations across SEFI and WEI (Fig. 1.). Mice were trapped using Sherman Live traps and had the last 1cm of tail tissue removed and stored in a buffer solution.

DNA samples were also sent to the University of North Carolina where they were compared using a Mouse Diversity Array and referenced to a set of genotypes from 200 wild caught and wild-derived strains of *M. m. domesticus*, *M. m. musculus* and *M. m. castaneus*. (Didion et al 2012). Heterozygosity of Farallon mice was compared with European House mice, and the geographic origin of Farallon mice was inferred from phylogenetic clustering. Possible anticoagulant resistance in the mice was assessed by examining Vkorc1 alleles, which encodes a protein critical for blood clotting. Mutations in Vkorc1 in rodents are associated with resistance to Warfarin, a first-generation anticoagulant. Several species of rodents are known to have resistance alleles, including *M. spretus*.

# **Non-target Species Risk Assessment**

During the period that non-toxic bait containing pryanine was available, attempts were made to quantify the level of exposure that might occur during a mouse eradication to western gulls, burrowing owls, salamanders and other species.

*Western gulls*

Following each bait broadcast, western gulls were allowed to naturally congregate and forage on bait pellets without any human interference. Over the course of the eight days that bait was available, daily surveys were conducted in an attempt to document instances of gulls consuming bait pellets and quantify the proportion of the population observed to be feeding on rodent bait. Personnel were stationed on Lighthouse Hill during the early morning and late afternoon hours to count the number of gulls present or feeding within baited areas.

As with mice, gulls which consume pyranine excrete feces which fluoresce under UV light. In an effort to further quantify the proportion of the gull population consuming bait, two fecal plots were demarcated one on the helipad and one on the gull roost west of Mirounga Beach (Fig. 2). Following the first bait application, the total number of fecal deposits was recorded daily, as were the number of deposits which tested positive for fluorescent dye. No monitoring was undertaken prior to bait application so naturally occurring rates of fluorescence ([Sztukowski 2011](#_ENREF_23)) were not established.

Pyranine can be used to detect not only primary but also secondary consumption ([Stephenson et al. 1999](#_ENREF_21)). In conjunction with ongoing research being conducted on the island, burrowing owls captured in mist nets were inspected for signs of the pyranine fluorescent dye. Owl fecal pellets were also collected and examined for UV fluorescence.

*Salamanders*

Cover boards were put out in the Marine Terrace study area in order to assess exposure of salamanders (Fig. 3). Boards were set out in October 2010, prior to the trial in order to allow salamanders some time begin using the boards. Non-toxic bait pellets containing pyranine were hand broadcast at ~18 kg/ha in known salamander habitat along half of the salamander cover board monitoring area along North Landing Trail (Fig. 3). Monitoring with a UV light underneath and around 100 salamander monitoring boards was completed three days after bait application to assess if any salamanders or invertebrates showed evidence of fluorescence that would indicate biomarker exposure.

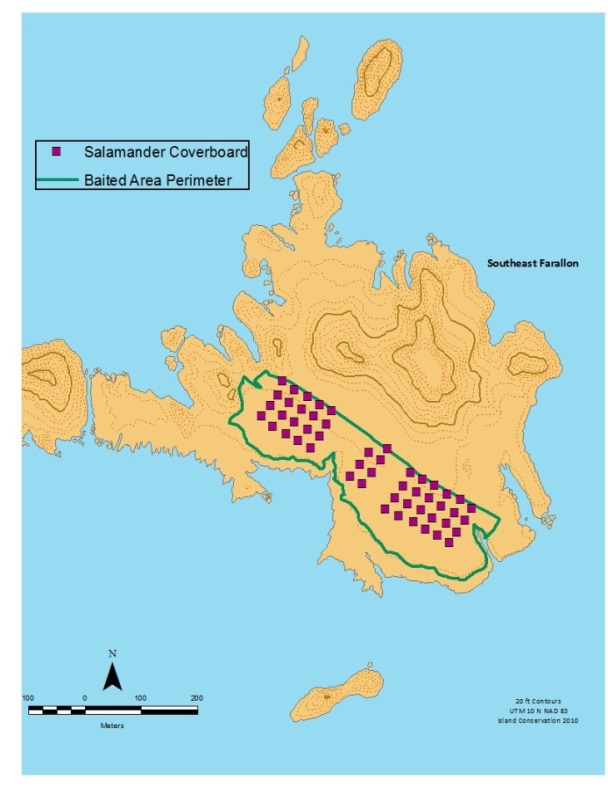


Fig. 3. Salamander cover board locations in relation to baited areas

*Other non-target species*

Observations of bait take or scavenging of mouse carcasses by other species were recorded.

*Secondary poisoning risks*

An evaluation of secondary poisoning risks was made by monitoring the fate of mouse carcasses positioned within baited areas. A varying number of carcasses were set out on a daily basis and checked daily thereafter. Western gulls have been identified as being particularly vulnerable to the use of rodent bait containing rodenticide because they are omnivorous scavengers and individuals of this species will be present on the South Farallon Islands during the time of year that a mouse eradication might be undertaken.

# **Use of Bait Stations to Mitigate Non-target Species Risk**

Two different bait station types housing non-toxic rodent bait were field tested on the Farallones to assess if they would restrict gulls from accessing and consuming bait. The Protecta (Fig. 4) is a commercially available bait station made of impact-resistant, injection molded plastic (Bell Laboratories, Inc., Madison WI). It can be staked to the ground for security. The box opens from the side for servicing using an Allen key wrench. Its dimensions are 6” x 5” x 2.5”. A second type of bait station was constructed solely for the purposes of the trial (Fig. 5). A PVC conduit box with PVC tube extensions on either side allowed two entry points for mice. The top of the conduit body unscrews for inspection and refilling with bait.

Ten Protectas and 10 novel bait stations were deployed on Southeast Farallon Island from November 8 – 17, 2011. Stations were evaluated in a paired test, with each pair 1m apart, and each pair of stations separated by 10m from adjacent pairs. Both bait stations were attached to redwood boards approximately 12 inches square and 2 inches thick, which secured them to the ground and made them more resistant to disturbance by gulls or pinnipeds. Bait stations were left out unbaited for two days to season them before being filled with 20g of non-toxic bait pellets (~20 pellets @ ~1g each). The non-toxic bait pellet used in the bait stations was brodifacoum (25D Conservation) because these were known to be palatable to Farallon mice.



Fig. 4. Protecta bait station (bait blocks depicted were not used in this trial)

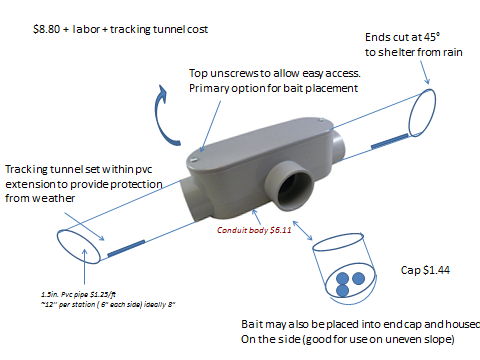
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Fig. 5. Novel bait station (developed by Island Conservation)

Acceptability of bait stations to mice was evaluated by two measures; mouse visitation and bait consumption. Mouse visitation was evaluated by placing tracking pads inside the entrance of each station. A tracking pad consists of a strip of felt moistened with peanut oil and oil based black ink and fastened to a length of white absorbent paper. Once a mouse enters the station and steps on the felt pad, its tracks are imprinted on the paper. Each day, the ink pads were inspected for mice tracks and collected. Bait consumption was quantified by weighing and recording the bait remaining on a daily basis. Bait was replenished to maintain 20g of bait, and new ink pads inserted daily to track mouse activity. Relative differences in acceptability between station designs were determined by having stations placed in pairs at each site.

To assess the ability of bait stations to exclude gulls, stations were placed at known gull roosts where gulls were roosting near Low Arch and Mussel Flats on the Marine Terrace of Southeast Farallon. Observations were made daily at a distance throughout the day to assess if gulls or other species were investigating or disturbing the stations or accessing bait pellets.

# **Camel crickets**

Several caves on SEFI are inhabited by the endemic Farallon camel cricket. Presence and general abundance of these crickets were noted for designing future invertebrate surveys. Non-toxic bait was hand-broadcast at similar densities as for salamanders inside Rabbit Cave where camel crickets are abundant. A UV spotlight was used the day after bait application to determine consumption of bait by camel crickets. In addition, four caves were surveyed for the presence of camel crickets. At each site, estimates were made of the number of individuals, the portion of the cave that harbored the majority of crickets, distance from the entrance, and their location (wall, ceiling, or floor).

# **Treatment of Caves**

Numerous caves, coves, and coastal features on SFI may require special attention during a mouse eradication. To investigate the extent and evaluate potential options for treating these sites, caves were visited and mapped using GPS equipment. Some rough measurements of the dimensions of the geographic features of some of the caves were also made.

# **RESULTS AND DISCUSSION**

# **Mouse abundance**

Out of 500 possible trap nights, 434 mouse captures were recorded. Trap success averaged 93% on all but the first night, when trap door setting sensitivities may have resulted in a lower trap success rate of 62%. A total of 250 different individuals were captured and marked in the trapping period in the 0.2 ha trapping area. Recapture rates of marked individuals on nights 2 through 5 were: 35%, 40%, 56% and 66%, respectively. Mice were extremely abundant and easily trapped, likely due to a combination of high population levels and a scarcity of other food resources. Mice were commonly seen foraging throughout the daylight hours, as well as at night, but traps were only left open at night.

While final density estimates have not been calculated, preliminary analysis suggests densities of mice of up to 1297 per hectare in the study area at this time of year. Mouse densities at these levels have only rarely been reported elsewhere and usually only during plague-level irruptions in a few locales world-wide. Abundance levels found on SEFI are ten times greater than reported densities in most island or mainland environments. The likelihood that mice were hungry and readily trappable on the island during this time of year bodes well for an eradication attempt undertaken during this period, as it is more likely they will accept bait under stressed and food deprived conditions.

While specific mouse home-range studies were not conducted during the trial, the five-night mark-recapture study resulted in 101 mice that were captured at least twice, and some as many as five times. The mean maximum distance moved for mice captured two or more times was 11.7m. Of recaptured mice, 82% moved less than 16m between most distant captures. A further 10% of recaptured mice moved as much as 24m. Only six mice moved more than 35m, and the longest recapture distance was 43m. While the size of the trapping grid (45m) may have biased some of the longer ranging results downward, 95% of the maximum distances moved on SEFI are within the expected diameters (10-29m) for reported mouse home ranges reported for house mice in another temperate island environment ([Pickard 1984](#_ENREF_19)).

Monthly monitoring of mouse activity is ongoing.

# **Mouse Reproductive Status**

The live-trapping of over 900 individual mice on SFI during the November 1-22 period revealed no pregnant females and only three males that were scrotal and five that were partially scrotal. Thus while some breeding may occur during this time of year, it would be considered a rare event based on our results. This also bodes well for an eradication attempt during this time, as it means that the risk of juvenile weanling mice being missed by any of the bait application events is low.

# **Pyranine Persistence in Mice**

During the lab trials, all mice that were fed the pyranine-infused bait tested positive for external sign of fluorescence (on mouth or anus) under UV exposure after 24 and 48 hours. On the third day (72 hours) however, one of the twelve mice tested negative for the presence of pyranine. By day four (96 hours) ten of twelve mice showed no external evidence of fluorescent dye. Although necropsy was available for the field trial, based on the results of the pyranine trial, trapping field to assess levels of exposure during the field trial was concluded within 72 hours of bait broadcast to avoid false negatives.

# **Bait Palatability and Food Preference**

Mice in the bait preference trial consumed an average of 3.8g of food each day, with individual consumption ranging between 2.7g and 4.7g. Consumption was on average about 20% of their body weight each day. All ten mice included in the trial preferred bait pellets over the natural food items provided. Preference for rodent bait also increased over the course of the trial from 50% on the first day to 63% and above on day two and for the duration of the study. Over the course of the trial, bait pellets on average constituted 62% of mouse diet (by weight) with naturally occurring foods making up the remainder.

Opportunistic observations made of mice after food choices were first presented showed that rodent bait was usually eaten first. In only one of ten instances, was coleopteran larva eaten first. Visual observations also confirmed that bait pellets were easily picked up, handled and carried by mice. This was also noticed in the field where pellet caching was seen at burrow entrances. Overall, bait trial results indicated that the bait being considered was readily accepted by the mice, and that all mice had consumed the non-toxic equivalent of an LD50 within 48 hours.

# **Bait Availability**

Monitoring of bait availability transects showed that after the first application at 18kg/ha, bait remained available to mice for at least four nights. This period of time has been the target exposure period for past rodent eradication projects that used second-generation anticoagulants. However, the rate of bait disappearance appeared to accelerate after Day 3 and on the fourth day after bait application, bait had disappeared from all but one transect (Figs 6 and 7). Bait was removed at an average rate of 3.6kg/ha/day, with daily uptake rates per plot ranging from 1.6-6.3 kg/ha/day over five days.

Rates of bait disappearance observed after the second application were much higher with most bait gone from availability transects in both areas the day after its application. Bait disappeared overnight from many transects monitored in Area B where bait was applied at 9kg/hand. Bait persisted a little longer in Area A where bait was applied at 18kg/ha but still disappeared within two days on most transects. Mouse abundance in Area B was an order of magnitude higher than in Area A and the increased rate of bait disappearance observed in Area B is considered attributable to mice. Bait within the gull exclusion cages established in Area B also disappeared in less than two days ruling out gulls as a factor strongly influencing bait disappearance in this area.

**Fig. 6. Bait availability over time in Area A on SEFI following two applications of rodent bait (1g pellets) at 18kg/ha across a 3 ha trial area.**

In Area B, bait disappeared from within gull exclusion cages after both applications at a significantly faster rate than bait outside (*t* = 4.47, *df* = 10, *p* < 0.01). The opposite trend was observed in Area A (*t* = -5.06, *df* = 10, *p* < 0.01) suggesting that consumption of bait by gulls did contribute to bait disappearance there. Observations of greater numbers of gulls foraging in Area A support this view. By the time of the second application, individual western gulls roosting along the Marine Terrace had clearly learnt to identify rodent bait as a food item and were observed foraging in increasing numbers in both areas but most intensively within Area A. Although sample sizes are considered too small to be representative, results from Area A indicate that it is possible that gulls could consume a significant amount of rodent bait if no gull hazing is undertaken. Consumption of bait by gulls appeared to increase over the course of the trial and increased consumption by gulls may partially explain the greater rates of bait disappearance observed after the second application.

**Fig. 7. Bait availability in Area B over time on SEFI following two applications of rodent bait (1g pellets) at 18kg/ha and 9kg/ha across a 3.2ha trial area.**

The study area was located in a favored roosting site for western gulls and the impact of gulls was very different between the two baited areas. Consequently, our results may not be representative of the influence gulls could have during a mouse eradication. Our results suggest that the impact of gulls on bait availability is likely to vary across the island and over time. Nevertheless, there is a risk that gulls could reduce the amount of bait available to some mice. The potential increased risk that this poses to the proposed eradication is another valid reason for implementing a hazing program as a mitigation strategy during a mouse eradication attempt.

# **Mouse Biomarker Exposure Rates**

The trap results indicated a very high rate of exposure to bait in the core trapping grids. Four trap nights were conducted in each of the two core trap grids with areas A and B. On the trapping grid within Area A, 100% of trapped mice had consumed bait as evidenced by the presence of pyranine after each of the two applications at 18kg/ha. A total of 13 mice were captured in grid A, amounting to 2% trapping success.

On the trapping grid with Area B mouse trapping success rates were much higher, with 25 mice captured after the first application (6.5% trap success) and 129 mice captured after the second bait application (32% trap success). All 25 mice trapped after the first bait application (18kg/ha) tested positive for fluorescent dye (100% exposure) (Table 1). After the second application at 9kg/ha, five of the 129 mice trapped on the core trapping grid and one mouse caught within the baited area but on the immigration transect showed no evidence of fluorescent dye (Table 1). The overall rate of exposure recorded from within Area B was 97%.

**Table 1 Mouse Trap Results for Biomarker Presence**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Trap Area** | **# Traps Set** | **# Mice** | **# Positive** | **% Positive** | **# Negative** | **% Negative** |
| Core Grid A Nov. 12 | 200 | 2 | 2 | 100 | 0 | 0 |
| Core Grid A Nov. 13 | 200 | 2 | 2 | 100 | 0 | 0 |
| Core Grid A Nov. 17 | 200 | 3 | 3 | 100 | 0 | 0 |
| Core Grid A Nov. 18 | 200 | 6 | 6 | 100 | 0 | 0 |
| **Core Grid A - Total** | **800** | **13** | **13** | **100** | **0** | 0 |
| Core Grid B Nov. 12 | 200 | 16 | 16 | 100 | 0 | 0 |
| Core Grid B Nov. 13 | 200 | 9 | 9 | 100 | 0 | 0 |
| Core Grid B Nov. 17 | 200 | 32 | 31 | 97 | 1 | 3 |
| Core Grid B Nov. 18 | 200 | 97 | 93 | 96 | 4 | 4 |
| **Core Grid B Total** | **800** | **154** | **149** | **97** | **5** | **5** |
| Inner Immigration A | 40 | 16 | 16 | 100 | 0 | 0 |
| Inner Immigration B | 40 | 17 | 16 | 94 | 1 | 6 |
| Outer Immigration A | 16 | 11 | 1 | 9 | 10 | 91 |
| Outer Immigration B | 40 | 25 | 0 | 0 | 25 | 100 |

As no barrier existed to prevent mice from immigrating into baited areas, transient mice could have been trapped before being exposed to bait. The probability that immigration occurred is supported by the huge increase in the number of trapped mice in Area B on the night two after the second application. However, it is also possible that resident mice did not have access to bait or chose not to eat it. Consumption by con-specifics and gulls is likely to have reduced the availability of bait to resident mice. In an eradication operation competition with con-specifics will be eliminated after the first application of bait, but based on our results, gull consumption can be expected to increase overtime unless hazing is undertaken.

Palatability of rodent bait was confirmed by the captive choice study and the high rates of bait consumption observed during the field trial and it is considered unlikely that mice chose not to eat the bait especially as the population was likely food limited during the trial. Despite the capture of unexposed mice the results indicate that application of rodent bait at the rates used in the trial would have a high likelihood of exposing all mice on the South Farallon Islands.

# **Mouse DNA and Genetic Analysis**

A total of 100 DNA tissue samples were collected during the trial, with 50 from each of SEFI and WEI. These samples have been stored for future analysis. Genetic analysis was conducted on the 25 House mice (11♂, 14♀) collected from around the residential area on Southeast Farallon Island. Diagnostic alleles assigned the subspecific origin of the Farallon mice to be overwhelmingly of *M. domesticus* origin (Fig. 8) ([Didion et al. 2012](#_ENREF_7)).



Fig. 8. Origins of introduced house mice found on Southeast Farallon Island

Heterozygosity was higher in Farallon mice than European mice (9.3% vs 8.8%), with no evidence of inbreeding, which suggests that diversity was maintained by rapid population expansion following colonization. The geographic origin of Farallon mice, inferred from phylogenetic clustering revealed two common lineages. Maternally, Farallon mice belong to the BritIsl.5 haplotype group, which is found in northern UK, Germany, Scandinavia and former British colonies and differs only slightly from classical inbred strains. Paternally, Farallon mice cluster with samples from the Mediterranean. Thus, Farallon mice appear to be a mixture of two European lineages ([Didion et al. 2012](#_ENREF_7)).

Vkorc1 encodes a protein that is critical for blood clotting. Mutations in Vkorc1 in rodents are associated with resistance to Warfarin, an anticoagulant that is used as a rodenticide. Several species of rodents are known to have resistance alleles, including *M. spretus*. It was recently shown that *M. m. domesticus* from the Mediterranean (specifically Spain) have received *M. spretus* resistance alleles by adaptive introgression. Analysis showed that Farallon mice are of Mediterranean ancestry in the region containing Vkorc1. Sequencing of Vkorc1 in all Farallon mouse samples revealed no evidence of resistance alleles. It was concluded that there is no known genetic barrier to an eradication utilizing a rodenticide for Farallon mice ([Didion et al. 2012](#_ENREF_7)).

# **Non-target Species Risk Assessment**

*Western gulls*

The total number of western gulls was highly variable during the trial period, ranging from day to day from approximately 500 to 4000 individuals. Numbers also increased over the trial period. The population is thought to shift sporadically from mostly non-breeding, intertidal-roosting gulls in November to a larger percentage of territorial, breeding gulls later in December and January. Breeding birds begin to spend more time on potential breeding sites throughout the island in advance of their breeding season, with the earliest egg-laying dates generally occurring in late April, when up to 17,000 gulls may be present on the island. Daily gull counts continue to be conducted by PRBO staff.

A total of 324 hours of visual observations of gull foraging within the baited area were recorded. Over the first 24 hours after the first application fewer than 12 western gulls were seen foraging on bait in a few small areas. By the second day, 188 gulls were observed consuming pellets in baited areas and by the third day, 233 gulls were seen consuming pellets. On days four and five, the fraction of foraging gulls dropped below 12% of the total number of gulls present within the Marine Terrace area, perhaps due to a paucity of remaining bait (Fig. 9). Following the second application of bait, the number of gulls foraging on bait grew from 22% to 43% of the gulls present in the study area, likely in response to the second bait application. On average, 27% of the gulls present on the Marine Terrace were observed foraging on bait over the course of the eight days that bait was available within the study area.

On average, 27% (range 0 – 67%) of gull feces monitored with a UV spotlight following the application of rodent bait showed signs of pyranine. While this figure agrees with the relative proportion of gulls seen foraging on bait, because we did not establish a baseline to determine naturally occurring fluorescence, it is possible that this method overestimated the proportion of the population exposed.

The significantly higher rates of bait disappearance observed outside of gull exclusion cages in Area A together with our observations of gulls highlight the potential influence that gulls could have on bait availability for mice. The increase in the number of gulls foraging on rodent bait over the course of the trial suggests that identifying rodent bait as a food source was a learned behavior. Additional gulls appeared to be drawn in to an area because of the presence of foraging gulls. A hazing program should aim to attempt if at all possible to prevent any gulls from foraging on bait to limit the potential for behavioral transmission. Most gull foraging activity observed during the trial occurred in the first two hours after sunrise and in the two hours preceding sunset. This pattern could be exploited in a gull hazing program.

Fig. 9. Percentage of gulls in study area observed feeding on bait

*Burrowing owls*

A total of 10-12 burrowing owls were likely present on the islands during the November trial, many of which had been captured and banded and/or fitted with a radio-transmitter as part of ongoing research. Two owls were captured in mist nets and examined under UV light for exposure to the fluorescent dye, but neither individual showed any sign of pyranine. A total of 26 fresh burrowing owl casts were also collected from 10 locations within and near the study area both before and after bait application. None showed any that would have indicated exposure to pyranine. However, based on other studies ([e.g. Stephenson et al. 1999](#_ENREF_21)), it is likely that during a mouse eradication burrowing owls would be at risk of exposure to rodenticide by consuming dead or dying mice. The results of our study with regard to burrowing owls are considered inconclusive.

*Salamanders*

Inspection of cover boards before and after the application of bait revealed just six salamanders and none of these showed any signs of having being exposed to rodent bait. A further five salamanders were captured outside of the area where bait was applied and these too showed no signs of exposure. Invertebrates under or near cover-boards were also examined also with no evidence of exposure.

*Other species*

Although invertebrates were seen consuming bait, no consumption by other non-target species was noted. However, raptors and corvids present during a mouse eradication should still be considered to be at risk.

*Secondary poisoning risks*

Scavenging of mouse carcasses was observed during the trial. Eighteen of 23 carcasses set out within Area A and B disappeared within five days () of being placed. Although most scavenging of carcasses appeared to be by other mice, some mouse carcasses could have been scavenged by western gulls or ravens (*Corvus corax*).

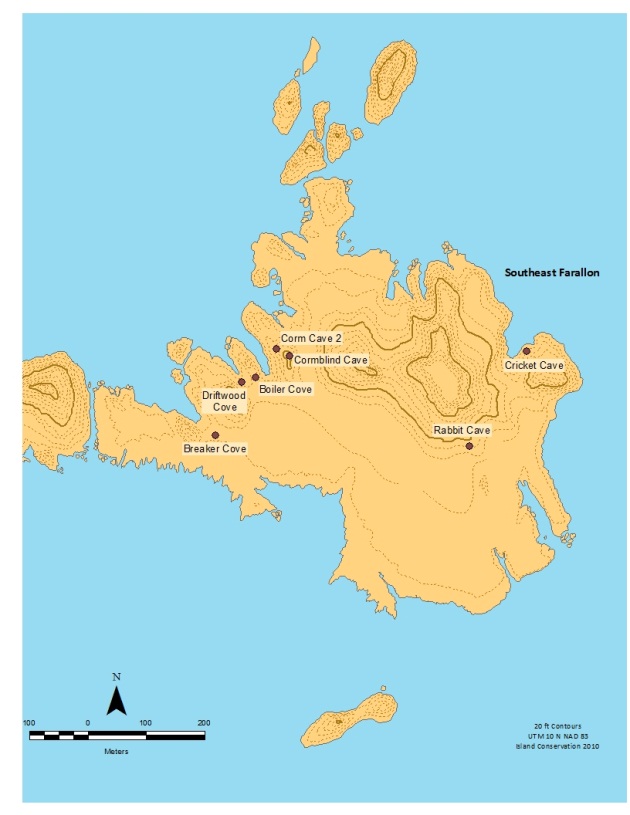


Fig. 10. Caves and coves inspected during the November 2010 trial and recorded on GPS units

# **Use of Bait Stations to Mitigate Non-target Species Risk**

As evidenced by the tracking rates and bait consumption observed, both bait stations tested were readily used by mice and no discernible difference could be detected in the use of either type of station. Similar tracking rates and levels of bait consumption were recorded between the two models of bait station tested. No evidence for neophobia was observed. Both stations were effective at protecting bait from rain or wind driven spray.

No observations were made during the trial of gulls or other non-target wildlife taking bait from bait stations and it is concluded that both station types would be effective at excluding potential non-target species. Attaching stations to redwood boards was effective at eliminating potential disturbance by gulls or pinnipeds. In several cases, elephant seals were observed crawling over bait stations, yet these stations remained intact and upright. Once again both bait station designs performed equally in this regard. Fixing bait stations to boards allowed stations to be readily moved around whereas this would have been more difficult with other proposed methods such as rock anchors.

In summary, both bait station types trialed were readily used by mice and were effective at excluding non-target wildlife and it is considered that either design could be used during the proposed eradication. However, if bait stations are to be used as a secondary method in an eradication attempt, it is recommended that consideration be given to the additional operational risk that this entails. Using different methods for bait application adds complexity to operational planning and creates a greater risk of gaps in bait coverage between areas where the application method is different. Bait station operation span a greater time period than those where bait is hand or aerial broadcast adding complexity to the timing of an operation.

It is recommended that a gull hazing trial be undertaken on the South Farallon Islands to explore further mitigation options for western gulls.

# **Camel crickets**

Surveys with a UV spotlight after rodent bait had been spread in Rabbit Cave indicated that camel crickets did ingest bait. Farallon camel crickets are not considered at risk because invertebrates do not have the same blood clotting system as vertebrates and are generally not susceptible to anticoagulants ([Shirer 1992 in Ogilvie et al. 1997](#_ENREF_18)). Experiments exposing other Orthopterans such as locusts (*Locusta migratoria*) ([Craddock 2003](#_ENREF_5)) and tree weta (*Hemideina crassidens*) ([Morgan and Wright 1996](#_ENREF_16)) to second-generation anticoagulants illustrate the lack of susceptibility. Camel crickets are also considered an unlikely pathway for secondary poisoning of other native wildlife except perhaps mice because they are only found in caves.

Interestingly crickets that had ingested the non-toxic rodent bait containing biomarker were easier to see and census with the UV light than traditional methods employing regular head lamps. In some cases estimates of cricket abundance quadrupled; it was easier to see crickets fluorescing under the UV lights. The number of crickets estimated from each cave prior to UV inspection were: Rabbit Cave: 100; Spooky Cave: 300-500; Northern Corm Blind Cave: 100; Cricket Cave: 1100; Small Shubrick Cave: 30. Data from these pilot surveys will inform a long-term camel cricket monitoring program, and distribution and abundance will be assessed before and after the proposed mouse eradication attempt.

# **Treatment of Caves**

Fig. 10 shows a map of the caves that were visited and mapped during the trial. Other cave locations may still need to be inventoried prior to operational planning. Caves have the potential to harbor mice and it is recommended that rodent bait is spread within caves during a mouse eradication attempt. An inventory of the cave systems should be made and this should be used during implementation of a mouse eradication to ensure all potential mouse territories are targeted.

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