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

Title:

Field trial using biomarker-treated placebo formulations of two commercial rodenticide baits to determine the optimal broadcast sowage rate to insure that all mice *(Mus musculus)* in the treated area are exposed to the bait

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Cooperator:

US National Park Service, Hawaii Volcanoes National Park

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June 20, 2013

Project ID:

QA-1857

Executive Summary:

1. We conducted field trials using placebo (no toxicant) formulations of 2 commercial rodenticide pelleted baits to determine if free-ranging mice (*Mus musculus*) consumed bait broadcast on the ground and to determine the optimal broadcast sowage rate (bait density) to insure that all mice were exposed to bait.

2. We hand-broadcast biomarker (Demethylchlortetracycline HCl (DMCT))-treated Ramik® placebo rodent bait pellets and Rozol® placebo rodent bait pellets at 2 application densities and trapped and examined mice for evidence of bait consumption.

3. The rodent bait provided were based on two commercial pellet formulations without the toxicant or the biomarker used in the labeled commercial formulation. The commercial formulations are Ramik Green® and Rozol® but the products tested were significantly different from the labeled products. Thus, the products tested herein are referred to as Ramik® bait pellets and Rozol® bait pellets.

3. Each baiting regime (2 baits, 2 application rates) was replicated 3 times at different sections of the 300-ha study site over a 5-month study period.

4. We determined that free ranging mice in the arid insular coastal habitat where the study was conducted readily consumed Ramik® bait pellets and Rozol® bait pellets broadcast on the ground. Both bait formulations were consumed by >95% of the mice trapped post-treatment .

5. Generally, more than 50% of broadcast baits disappeared after 4-5 days after initial baiting and less bait remained on the ground at the lower application rate for both bait types. Two consecutive broadcast application of bait spaced 8-14 days apart would be recommended under operational rodenticide baiting to insure that adequate quantities of palatable baits are available for the entire population.

6. If mice are breeding during an eradication operation, broadcasts should be done 12-14 days apart to allow young mice to emerge and begin feeding while bait is available.

7. Two applications of less than 22.4 kg/ha should be used to effectively target mice. Under the lower broadcast rate (11.2 kg/ha), the majority of Rozol® bait pellets were not available after 3 days for several of the treatment plots so a higher application rate would be needed to provide bait for a longer period. For the Ramik® bait pellet trials, more than 30% of the bait persisted for 5 days at application rates of at least 14 kg/ha. The ideal application rates would be more than 14 kg/ha but less than 22.4 kg/ha given the mouse populations in the study area.

8. We found some evidence that feeding increased after the first broadcast, suggesting that mice became accustomed to the novel food items after being exposed to bait for a week.

Citation:

Pitt, W. C., R. T. Sugihara, and D. K. Foster. 2013. Field trial using biomarker-treated placebo bait to determine the optimal broadcast sowage rate to insure that all mice *(Mus musculus)* in the treated area are exposed to the bait. QA-1857 Final Report. USDA, APHIS, WS, NWRC. Hilo, HI. 43 pp.

Introduction:

Rodents are very successful invaders and adaptable colonizers of island ecosystems worldwide (Adler and Levins 1994, Burbidge and Morris 2002) and have severely impacted the native floral and faunal resources of the Hawaiian Islands (Atkinson 1985, Scott et al. 1986, Buckle and Fenn 1992, Hadfield et al. 1993, Lindsey et al. 2009). While most mitigation measures have been directed against rats (*Rattus spp*.), depredation by non-commensal populations of house mice (*Mus musculus*) can be an equally significant threat to native conservation areas (Sugihara 1997, Courchamp et al. 2003, Jones et al. 2003, Cuthbert and Hilton 2004, Wanless et al. 2007, Hess et al. 2009).

Most insular rodent eradication projects have used brodifacoum, a second generation anticoagulant, because it is perceived to have higher efficacy (toxicity) against rodents, thus supposedly insuring a greater chance of success within a shorter period of time (Brown 1993, Taylor and Thomas 1993, MacKay et al. 2007). Increased awareness of direct and secondary non-target poisoning resulting from brodifacoum use in terrestrial ecosystem recovery programs has limited its use in many areas in favor of first generation chemicals such as diphacinone or chlorophacinone (Eason et al. 2001, Gilles et al. 2006, Hess et al. 2009, Pitt et al. 2010). Although brodifacoum (EPA Reg. No. 56228-36) is registered in the United States (Section 3) for conservation purposes, it has not been licensed for use in Hawaii's natural areas due to concerns about its persistence and toxicity to non-target species.

Investigators have documented the field efficacy of bait station and broadcast applications of diphacinone baits (Ramik Green®) against rats and its lower non-target hazards in conservation areas (Dunlevy et al. 2000, Nelson et al. 2002, Spurr et al. 2002, Spurr et al. 2003, Eisemann and Swift 2006, Johnson et al. 2005, Gilles et al. 2006, Witmer et al. 2006). A fish flavored compressed cereal grain bait pellet containing 0.005% (AI) diphacinone (EPA Reg. No. 56228-35) is approved for broadcast application in conservation areas in the United States (Section 3), including Hawaii.

Broadcast application of Rozol® chlorophacinone (0.01%, 0.005%) is approved for control of voles (Microtus spp.) in orchards and other field rodents in rangeland in California, Oregon and other US mainland states. Rozol® chlorophacinone (0.005%) block (EPA Reg. No. 7173-243) and pellet (EPA Reg. No. 7173-151) baits are registered (SLN) in Hawaii for use in bait stations and placement in ground burrows for rats and mice in tropical nuts, fruits and seed field crops (corn, soybean). As compared to diphacinone, higher efficacy (40-100%) was reported in laboratory bioassays of 0.005% chlorophacinone baits (Rowe and Redfern 1968, McCann and Matschke 2000, McCann 2000, Witmer 2007, Pitt et al. 2010). A higher mortality rate was observed in a shorter bait exposure period (7-10 days) than with feeding trials with the diphacinone baits. A broadcast application use pattern of chlorophacinone pellet baits is presently being considered by federal and state agencies for potential conservation use in Hawaii, pending completion of data sets on efficacy and non-target risks.

At a few targeted restoration sites in Hawaii, both rats and mice cohabitate and on at least two drier sites, Kaena Point Natural Area Reserve and Kahoolawe Island, mice are the sole or predominant rodent species found (KIRC 1998, Young et al. 2009). The current diphacinone broadcast application label was

developed primarily to target Polynesian (*R. exulans*) and roof rats (*R. rattus*), the major rodent species of concern in Hawaiian conservation areas (Swift 1998, Tobin 1994, Eisemann and Swift 2006, Lindsey et al. 2009). Rozol® chlorophacinone pellets may be a viable additional bait choice to the sole diphacinone conservation labeled bait against mice in conservation areas in Hawaii.

Effective baiting strategies against mice in conservation areas utilizing broadcast applications of first generation multiple-dose anticoagulants (i.e. diphacinone, chlorophacinone) are critically lacking. Information on optimal bait density and number of applications to insure 100% acceptance of bait by mice is needed. For broadcast application of anticoagulant rodenticides to be effective, the bait must be applied in sufficient quantity and density so all target animals encounter and ingest a lethal quantity of bait over a number of days (Marsh 1986). The bait sowage rate (11.2 -14.0kg/ha (10.0-12.5 lbs./acre)), and 2 consecutive applications spaced 5-7 days apart for the current registered broadcast diphacinone bait were based on laboratory and field trials with wild rats (*R. rattus* and *R. exulans*) (Swift 1998, Dunlevy et al. 2000). Increased bait application density and a prolonged bait exposure period (minimum of 14 days) may be required to insure sufficient bait consumption to effect acceptable control of mice.

Generating data specific to mice for the two rodenticides already approved for field use patterns in Hawaii is the most viable option available for planned Hawaiian ecosystem restoration projects targeting mice. The uncertainties on broadcast bait density and number of applications to ensure 100% bait acceptance by mice for all anticoagulant rodenticides warrant that further investigations are needed for these compounds before any mouse eradication or control plans are launched.

The objectives of this field study are (1) to determine if free-ranging mice consume Ramik® bait pellets and Rozol® bait pellets broadcast on the ground and (2) to determine the optimal broadcast sowage rate (bait density) to insure that all mice are exposed to bait. We will measure the proportion of mice caught in a core baited area that consumed biomarker-treated placebo formulations of both bait types after an appropriate bait exposure period. Two consecutive applications, spaced 7-10 days apart, of each placebo bait formulation will be applied.

Methods:

Study site description

The study was conducted in a 300-ha coastal, dry/mesic section of the Kealakomo-Kalapana lowlands in the Hawaii Volcanoes National Park, Hawaii Island (Fig. 1&2) representing typical natural areas targeted for invasive predator removal ecosystem restoration in Hawaii. Topography is generally flat, with the substrate dominated by *pahoehoe* lava and numerous upheaval patches of rubbly *a'a*. Elevation of the study site ranged from near sea level to 50m inland. Mean monthly temperature averages 22-26 C with 15-47mm monthly rainfall recorded during the drier May-August months. The site is exposed to 40-80 kph winds on most days due to the topography and its proximity 300-700m to the sea coast.

The few patches of soil (ash) found in depressions, cracks or fissures supports native and exotic scrub vegetation (ohia (*Metrosideros polymorpha*), noni (*Morinda citrifolia*), guava (*Psidium guajava*), akia (*Wikstroemia sandvicensis*), lantana (*lantana camara*), aalii (*Dodonaea viscosa*), ulei (*Osteomeles anthyllidifolia*), uhaloa (*Waltheria indica)* and indigo (*Indigofera suffruiticosa*)) and grass understory (natal redtop (*Tricholaena spp.*) and broomsedge (*Andropogon spp.*)). Geography and dominant vegetation found in the area are described in detail by Williams (1990).

Below is a non-exhaustive list of vegetation encountered within the study area. Vegetation in general, and grasses in particular, were more lush and dense in the first and second replicates that in the plots chosen for the third replicate (plots 7,9,12,13; Fig 3). Fruit from guava, pukiawe, noni, and ulei were available the entire duration of the study.

Trees and shrubs:
ohia (*Metrosideros polymorpha*)
noni (*Morinda citrifolia*)
guava (*Psidium guajava*)
akia (*Wikstroemia sandvicensis*)
lantana (*lantana camara*)
aalii (*Dodonaea viscosa*)
ulei (*Osteomeles anthyllidifolia*)
uhaloa (*Waltheria indica*)
indigo (*Indigofera suffruiticosa*)
pukiawe (*Styphelia tameiameiae*)

Graminoid and forb understory:
broomsedge (*Andropogon virginicus*)
pili grass (*Heteropogon contortus*)
natal redtop (*Melinis repens*)
molasses grass (*Melinis minutiflora*)
japanese tea (*Cassia leschenaultiana*)
button sedge (*Fimbristylis cymosa*)
golden beardgrass (*Chrysopogon aciculatus*)
billygoat weed (*Ageratum conyzoides*)
swordfern (*Nephrolepis multiflora*)
Pohapoha (*Passiflora foetida*)

Numerous species of native and exotic birds are found within or adjacent to the study plots. Black noddy (*Anous tenuirostris melangensys*), Golden plover (*Pluvialis dominica*), Japanese White-eye (*Zosterops japonica*), Northern cardinal (*Cardinalis cardinalis*), House finch (*Carpodacus mexicanus*) and Barred dove (*Geopelia striata*) are common and an occasional Hawaiian owl (*Asio flammeus sandvicensis*), Hawaiian hawk (*Buteo solitarius*), and Hawaiian goose (*Nesochen sandvicense*) reported.

Mammals commonly recorded in the area include rodents (*R. spp.*, *M. musculus*), mongoose (*Herpestes auropunctatus*), feral pigs (*Sus scrofa*), and feral cats (*Felis catus*). Numerous fecal droppings of the latter two species have also been observed throughout the study area.

Plot selection

The four baiting regimes were each replicated 3 times within the 300-ha study area between March and July 2012 (Fig. 3). Each treatment replicate consisted of 4 distinct study plots that were baited at different time periods due to manpower limitations. A baiting regime (2 baits types and 2 application rates) was randomly assigned to each plot. Individual plots were separated by 200-400m to limit migration of mice between treatment plots. The plots were delineated with a handheld Trimble© GPS unit, each plot measuring 1.0 ha in size.

Bait procurement and biomarker treatment

Placebo bait pellets (Ramik® bait pellets -approximately 1.5gm/pellet, Rozol® bait pellets - 0.25-0.28gm/pellet) were prepared by the respective bait manufacturers (Hacco®, Liphatech®) without the active ingredient. Demethylchlortetracycline (DMCT) (0.05% w/w) biomarker was incorporated into the bait during the manufacturing process. DMCT is a tetracycline class antibiotic with excellent fluorescent properties that chelates with calcium tissues such as bones and teeth. Previous research at the Hawaii NWRC (National Wildlife Research Center) field station found DMCT to offer the best combination of palatability and marking performance capabilities for invasive house mouse in Hawaii (Pitt et al. 2012). Commercial-sized batches (600-720kg) were produced, shipped to the field station and stored in sealed plastic bags within a fiberboard box in a darkened ventilated room until used. Subsamples of each bait type were randomly collected and sent to the NWRC Analytical Chemistry Section to analyze for presence of potential trace active ingredient contamination.

Baiting regimes (treatments)

The Ramik Green® diphacinone label allows for broadcast application rates of 11.2 to 14.0 kg/hectare. A second application at a rate no higher than 14.0 kg/ha can also be made 5-7 days after the first application. Alternatively, a single application not higher than 22.4 kg/ha can be made when weather and logistics limit the application of bait. All existing Special Local Need labels for aerial broadcast of Rozol® chlorophacinone pellet bait targeting voles in orchard and rangeland, among other locations, have a single application of 11.2 kg/ha.

We evaluated placebo pellets of each of the bait formulations at the following baiting regimes:

Bait type	Broadcast application rate	Number of applications
Ramik® bait pellets	14.0 kg/ha	2
	22.4 kg/ha	2
Rozol® bait pellets	11.2 kg/ha	2
	22.4 kg/ha	2

The baiting regimes were based on registered recommended broadcast application rates for each bait formulation and the published higher bait density recommendations for mice based on their smaller home range size and movement patterns (Fitzgerald et al. 1981, Mackay et al. 2007). Two (2) separate bait applications spaced 7-10 days apart were conducted for each bait type and application rate to insure sufficient and continuous supply of bait was available to all mice in the area prior to trapping.

Broadcast bait application

The biomarker-treated placebo baits was hand broadcast by 4-5 personnel walking abreast at 5-m spacing and hand casting bait over a 2.5-m swath on either side of flagged parallel transects. Transects spaced 10 m apart were established in each plot to facilitate personnel access for hand bait application and also assist in placing and locating traps. Wire survey flags were placed at 10-m intervals along the length of each transect for guidance and served as reference markers for placement of traps. There was no clearing of vegetation and debris. Prior to actual bait application, all personnel practiced dispensing placebo pellets to simulate hand bait broadcast at the targeted application rate in an open field near the Hilo field station. The density of pellets broadcast on the ground was calculated and application techniques adjusted to achieve as uniform and as close to the target pellet density (Fig. 4) as possible prior to actual field site application. The target number of pellets per square meter corresponding to the different broadcast application rates are as follows:

		# of pellets/m^2 by application rate		
Bait type	Pellet wt. (gm)	11.2 kg/ha	14.0kg/ha	22.4 kg/ha
Ramik® bait pellets	1.5	----	1.00	1.50
Rozol® bait pellets	0.3	3.75	----	7.50

Each applicator carried a pre-weighed batch of bagged bait sufficient to cover the individually traversed transect segment (5-m swath x 100-m length) at the targeted bait application. Each applicator baited 4-5 transect segments per plot. All 4 plots were baited on the same day within a 2hr period and the procedure repeated for the second baiting 7-10 days after the initial broadcast baiting.

Fate of broadcast bait

The ability of mice to find and consume sufficient quantity of bait over the duration of the bait exposure period would be seriously compromised if significant amounts of broadcast baits became unpalatable or were consumed by non-target animals present in the treated area. We monitored the fate of broadcast bait pellets on the ground outside of the central trapping grid of each plot. Bait condition (weathering, hardness, mold) and fate (disappearance, or partial consumption by mollusks and other invertebrates) at 10 randomly selected locations was documented on the first and every other day for 7 days after each bait application. Individual pellets (2-8, corresponding to the respective bait density of each bait type) were placed on the ground at each sampling location within a 1 m^2 area to determine whether "palatable" bait was available throughout the 7-10 days exposure period of each broadcast application of bait.

Ten (10) 20cm x 20cm inked tracking tiles (Marten 1972) with 2-4 glued pellets/tile were placed on the ground in the same general sampling location in each plot to identify bait visitors and determine the proportion of baits taken by mice and other non-target consumers and monitored throughout the 7-14 days bait exposure period of the trial. Tracks left on the tiles were examined and identified by type (rats, mice, mongoose, ants, birds). Tracking tiles were discontinued after the first replicate due to dusty soil and debris contamination of the inked surfaces and inability to discern tracks in the windy study sites.

Digital Reconyx® IR trail cameras (4/plot) were staged at strategic locations in the plot to also identify target and non-target bait visitors and document bait fate. Cameras were directed at sowed pellets on the ground and operated for 6-7 days of each bait application. Cameras were checked daily, adjusted and data downloaded as needed.

Mice trapping

In mark-recapture depletion sampling activities there is the possibility of "edge effects" where unmarked mice immigrate from outside the treated area and are captured disproportionately along the edge of the sampling grids, Thus a core trapping area (60m x 60m) was established in the center of the 1-ha plot, 20 m inside from the edge of the baited area to increase the likelihood that trapped mice were fully exposed to the baits and were not immigrants from non-baited areas (Chelkowska and Ryszkowski 1967, Stenseth and Hansson 1979). A Sherman® (live) aluminum mice trap (52.5cm x 6.25cm x 16.25 cm) was placed at 10-m intervals within the core trapping grid (60m x 60m) of each plot, for a total of 49 traps. Enclosed Sherman® traps were used to minimize carcass predation by rats, mongooses or other predators commonly found in the study site. Trapping was initiated 4-5 days after the last bait application based on results of previously conducted laboratory trials that determined the minimum period of time needed after bait consumption to easily detect the DMCT biomarker and thus verify exposure to the bait (Pitt et al. 2012).

We broadcast small amounts of grated coconut (*Cocos nucifera*) within the trapping grid 2-3 days prior to actual trapping to familiarize mice to the novel bait. Traps were baited with coconut chunks (12-

15mm square), placed on the ground at each grid location, and maintained for 4 consecutive nights. Trapping for 4 nights allowed for the resident mouse population to be adequately sampled without substantial captures of new cohorts and immigrating juveniles/adults from adjacent untreated areas. (Tobin et al. 1996). Traps were strategically positioned under vegetation cover, in cracks or under rocky crevices whenever possible to reduce exposure to the elements. Traps were checked daily between 1000-1130h immediately upon arrival at the study site (2 hours commute by vehicle). Traps with captures were placed in a 30 gallon plastic trash bin where the animal was released, manually restrained and euthanized by cervical dislocation. Carcasses were individually placed in zip lock bags, which were labeled with the plot number, capture date, and trap location, placed in a Styrofoam® cooler and transported to the Hawaii Field Station. Although the traps used targeted mice, an occasional rat was captured and similarly processed.

Evaluation of bait consumption (biomarker detection)

We weighed, determined the age, verified the species and sex, and determine the reproductive condition of each captured mouse. We conducted an initial gross and UV-illuminated examination of the external surfaces of the mouth, face, anus, tail and feet of all mice for the presence of DMCT staining. We examined the exposed portion of the incisors, jaws and toe nails under a long wave (3150-4000 A) UV light for DMCT fluorescence. The lower and upper mandibles of mice exhibiting negative external fluorescence was excised, boiled in water, cleaned of flesh and air-dried. We pulled out the incisors from the mandibles with forceps and examined the inner basal tips for fluorescence. Mice were classified as exhibiting negative, weak or strong DMCT fluorescence of both the exposed and inner portions of the incisors (Pitt et al. 2012).

Experimental design and analysis

The proportion of mice in which DMCT fluorescence was detected was calculated for each bait type (n=2) and bait application rate (n=2) for each replicate (n=3). A randomized block design ANOVA (SAS 1990) was used to detect differences in the percentage of captured mice that consumed baits among bait type and application rate. Single factor ANOVA's using either replicate, plot, or trap night as a covariate were used to detect differences in mouse abundances and weights between treatments.

Results:

The field study was conducted from March-July 2012. Higher than average normal rainfall occurred during the first replicate (March-April) as compared to the second (May-June) and third (June-July) replicates. Dry conditions increased in June and July (Fig. 4). A total of 83.0mm, 18.0mm and 6.2mm precipitation was recorded during the 1^{st}-3^{rd} time periods (each 25 total days) respectively (Fig.4). The dry conditions during the third replicate were reflected in decreased average relative humidity recorded (69%, 70%, and 58% for replicates 1-3, respectively).

Upslope topography and NE trade winds exposure contribute to persistent windy conditions throughout the coastal study habitat. Winds averaged 12-40kph on most days with increased intensity as the day

progressed. Wind gusts of 50-55kph were frequent during replicates 1 & 3. May (replicate) was unusually calm with 18 days of <1.0kph average wind speed.

Generally, vegetation (mostly grasses) vigor and color were similar between replicates 1 & 2; however, the impact of reduced precipitation during period 3 was visibly evident in the drier condition of understory grasses. We did not quantify the availability of natural foods (seeds and fruits) in the study site. Observers noted that grass seeds and fruits of guava, noni, aalii, akia, and pukiawe were present during each period with ulei being more abundant during period 3.

Placebo bait application

Commercial-sized batches of 0.05% DMCT-treated placebo pellets (720kg- Ramik® bait pellets, 600kg-Rozol® bait pellets) were formulated by the bait manufacturers, received, stored at the field station and used in the study within 2.5 months of receipt. NWRC assays conducted prior to bait application indicated trace concentrations of 0.00014% AI of diphacinone (Ramik® bait pellets) and 0.00028% AI of chlorophacinone (Rozol® bait pellets). The manufacturer's equipment used to produce commercial rodenticide baits (0.005% AI diphacinone, 0.005% AI chlorophacinone) were cleaned prior to the production of the placebo baits. However, trace amounts of chemicals from prior production runs were expected to remain due to the logistically difficult and costly task of complete sanitation between production batches. Based on laboratory feeding trials (QA-1736) using placebo baits obtained previously from the same manufacturers, no adverse effects were recorded for mice consuming trace active ingredient-contaminated baits.

Four treatment plots were broadcast-baited by hand within a 2h period from between 9:00-11:00am on the same day for each replicate. A second application was applied 9-10 days after the initial baiting. This interval between consecutive bait applications, versus the recommended 5-7 days between applications of the commercial baits, was based on the observed availability of palatable bait remaining on the ground after 10 days exposure. Plagued by wind gusts up to 55kph during portions of the bait broadcasting, we made adjustments in the direction of bait hand-cast along each plot transect segment to insure uniformity of bait distribution in the treated plot.

Bait condition

Typical pellet inflation and deflation of extruded or pressed cereal grain baits with moisture and drying occurred with exposure post application (Koehler et al.1995, Dunlevy et al. 2000). Although soft and spongy after rainfall, Ramik® bait pellets quickly dried and retained their original texture and hardness within 4-5h in the dry, hot, and windy coastal habitat. Discoloration of the light-brown pellets was evident after 3-5 days with accelerated darkening during sustained sunny periods. DMCT naturally turns from yellow to tan to darker brown when exposed to sunlight. Pellets remaining from the initial bait application appeared blackish (moldy) when examined 12-14 days later and probably would not be palatable to mice. Rozol® pellets retained their shape and hardness over a longer period when exposed to precipitation; similar discoloration over time was noted.

Roaches (Blatteria), ants (Formicidae), slugs (Pulmonata), and crickets (Gryllidae) were observed feeding on both bait types starting at the first day post-bait application and throughout the 14-days monitoring period. Inked tiles with secured pellets used during the first period showed complete pellet removal (consumption) by mice within 24h, however, pellets depredated by invertebrates (mostly roaches and ants) disappeared gradually over 3-4 days.

Bait disappearance

Pellet disappearance rates for each bait type and application rate are displayed in figures 5-7. Continued removal of baits over time by rodents suggest that both bait types were highly palatable during the study period. Complete removal of pellets and partially eaten pellets were recorded at each monitoring station for 6-8 days and 7-12 days after the first and second bait applications. Dislodged pellets outside of the 1-m^2 sampling boundaries were left intact and not included in the count. Bait disappearance rates after the second application were directly related to subsequent trap success of mice in all treatment plots.

Differences in bait disappearance rates between bait types were not obvious. Baits were still available to foraging rodents for at least 5 days post application in all plots and at least 7 days in 10 of 12 trials. The 2 trials where all bait was consumed by day 7 occurred in plots with high mouse captures (65 and 78).

Remote camera bait monitoring

Besides invertebrates visibly observed feeding on the bait, remotely triggered cameras deployed during periods 1&2 recorded mice, rats and a single mongoose interacting with the bait pellets. Twelve (12) of 80 images of mice, 16/83 of rats (*R. rattus*) and 1/95 of mongooses showed direct interactions with baits, e.g., handling, removal, and feeding. Mice and rats were photographed only at night, whereas the one image of a mongoose chewing a Ramik® bait pellet occurred during the day. It was impossible to discern whether images of the visitors to the bait were of the same or multiple individuals.

Photo captures of feral cats (16) and pigs (7) and a single spotted dove (*Streptopelia chinensis*) showed no interactions with the broadcast baits.

Mice trapping

Sherman® (live) traps were set underneath natural (vegetation, rocks, logs) cover when available within two meters from assigned trapping grid locations. Trap sets on barren lava were placed within waxed juice cartons that provided the trapped rodents shelter from direct wind, rain, and solar heating. The majority of the shredded coconut pre-baited 3-4 days before setting traps had been consumed; however, coconut shreds were still visible on the ground in plots 7, 12, and 13 when traps were set. Coconut chunk trap baits were replaced after 2 nights or sooner if missing, dried or discolored (mold) in appearance. Depletion trapping was initiated 12 days following the last of the two bait applications and 21 days after the first application. Traps were maintained for four days.

We conducted a total of 2352 trap nights (live) during the study: 49 traps per treated plot, 4 treatment plots, 4 consecutive trap nights, 3 replications. A total of 485 mice and 3 black rats were captured and removed (depletion sampling) from the plots (Table 1). The nightly capture per unit effort (CPUE) was approximately 1 rodent for every 5 traps set and varied by plot and night. Depletion sampling failed to "trap-out" the treatment plots (Fig. 8). CPUE trends suggested that house mouse abundance within the treatment plots ranged from about 150 to 300+/ha. There were not significant differences in the quantity of mice captured in each treatment (ANOVA F-ratio = 2.562, p 0.138). However, there were significant differences in the number of mice captured in each replicate. Significantly fewer mice were trapped following third baiting replicate (July, 59 mice from 4 plots) than following either the first (April, 243 mice) or second (June, 181 mice) replicates (ANOVA F-ratio = 24.978, p 0.002).

House mouse whole body weights varied significantly between both plots (F-ratio = 5.486, p = 0.020) and treatments (F-ratio = 8.929, p = 0.00001). Average mouse body weights per plot ranged from 10.29–14.28g with the heaviest mice captured in plot 9 (table 2). Average mouse weights per treatment ranged from 11.44 to 12.95 and did not vary significantly between nights (table 3, F-ratio = 0.85426, p = 0.356). The smallest mouse captured in Sherman® traps weighed just 4.2 grams whereas the largest was a 23.6 g pregnant female. Late-term pregnant mice, determined via external abdominal palpitations, accounted for 37 of 276 (13%) captured females. All of the mice greater than 16 grams were females, many of which were pregnant (Figure 17). During late summer (July, replicate 3) a greater proportion of mice were heavier than those captured in the spring (April and May, replicates 1 and 2) and many of these larger mice were pregnant females (Figure 18).

There were no significant differences in mouse sex ratios among treatments (χ^2, p = 0.941), however, consistently more female mice (57%) were captured than males (43%) overall (χ^2, p = 0.002). This trend toward higher female capture rates was largely driven by a 2:1 female to male capture rate on the first trapping night (Table 3, chi-square p = 0.07 sex ratio x night). Three juvenile black rats, 2 males and 1 female, were captured within the Rozol 11.2 kg/ha treatment plots on April 12 and 13.

Insects, predominately ants and roaches, several moths and thrips, and a few centipedes, also sought refuge inside the live traps and on the coconut baits. On a few occasions the live traps were disturbed and moved a short distance away from its original location during the night, probably by rats, mongooses or pigs. One trap was destroyed by a feral pig and showed distinct teeth markings. The trap was found bent with closed doors and contained only the chunk coconut bait.

Bait Acceptance (biomarker detection)

DMCT fluorescence was readily visible in mouse and rat incisors and in the bones of the furred limbs and feet. For the majority of mice an external exam was sufficient to observe DMCT fluorescence in the exposed incisors and the bones (through the skin). When DMCT marks were either very weak externally or negative, the incisors were removed and observed with their respective cleaned mandible

for fluorescence. Fluorescent marks were scored subjectively as strong, weak, or negative based on the concurrence of three technicians experienced with tetracycline class rodent biomarkers.

There were no evident differences between the treatment groups in the proportion of marked captured mice by either bait type (Ramik® and Rozol® bait pellets) or sowage rate (11.2, 14, and 22.4 kg/ha)(ANOVA F-ratio 2.192, p = 0.167). Ninety six percent (465 of 485) of all captured mice were positively marked for DMCT, as well as all three rats captured (Table 1), suggesting high acceptance by rodents of the two placebo bait formulations. Both Ramik® and Rozol® marked mice at a similar high rate of approximately 70-100% of animals captured on any given night at both sowage rates. Marked mice were equally abundant on the fourth night of trapping as on the first night (day1 marked 96.7%, day4 marked 96.5%)(Figure 12). Mice without DMCT markings (negative) occurred at nearly the same rate of ~5 % in the four treatments (Table 4). Five to twelve percent of captured mice in each plot were positively marked internally and not externally - DMCT fluorescence was only observable after pulling and cleaning the incisors. Twenty-two to sixty percent of mice with DMCT marked incisors also had DMCT marked bones that were observable externally on intact mice (Table 4).

Spatial Capture Patterns

House mouse capture locations within the treatment plots have been mapped so that patterns, if they existed, could be discerned (Figs. 13-16). Captures, DMCT marking intensity, and unmarked mice occurred randomly across the trapping grid with a few exceptions. Traps set on bare exposed lava seemed to have poorer catch success than traps set under or against vegetative or other natural cover (personal observation).

Almost half the Sherman® live traps (24 of 49) were located along the outer edge of the 60x60m sampling grid (Fig. 3). The unmarked mice depicted in figures 13-16 were captured at either edge or interior locations and the results are summarized in table 4. Eight unmarked mice were captured in edge traps (47% actual, 49% expected) and nine unmarked mice were captured in interior traps (53% actual, 51% expected). No trapping "edge effects" were encountered (p = 0.808, chi-square) during the 4 days of trapping. Unmarked mice were captured on every trapping night at an occurrence rate of 1.8 to 3.7 % of total catch (Table 5). Capture rates of unmarked mice did not increase during the removal trapping effort (3.7% night 1 vs. 3.5% night 4).

Discussion:

The underlying requirement of a successful operational pest eradication program using toxic baits is that all targeted individuals are exposed to and consume bait. The baiting regime must insure that palatable baits are available to the targeted organism(s) over the required bait exposure period to effect control. In our case we targeted wild non-commensal house mice. In this field study we evaluated non-toxic formulations of two commercial grain bait pellets to 1) determine acceptance of broadcast bait by free-ranging mice and 2) determine the optimal broadcast sowage rate (bait density) to insure that all mice

are exposed to bait for a recommended multiple-feeding exposure period of 7-8 days (Swift 1998, Pitt et al. 2011).

We determined that free ranging mice in arid insular coastal habitats readily consumed Ramik® and Rozol® bait pellets broadcast on the ground. We did not detect any significant differences in the proportion of trapped mice consuming placebo baits (94-97% marked) by either bait type or sowage rate (11.2, 14, and 22.4 kg/ha), at mouse densities estimated to be as high as 300/ha (ANOVA, $p = 0.167$). Both bait types tested were equally palatable to mice in the dry coastal habitat of the study.

Sowed baits continued to disappear during the exposure period suggesting that mice were consuming palatable baits until at least 10 days post-application and perhaps longer if mice cached baits. Baits became discolored after ten days exposure to sun and rain and drying, and many individual bait pellets had signs of mold after which they were probably not palatable to mice. Darkened baits often had yellow centers indicative of surface DMCT photo-degradation. Our laboratory experiments revealed that aged, browned DMCT (0.05% wt/wt, fresh DMCT is a yellow powder) was still capable of imparting fluorescent marks on mouse incisors, thus baits aging in the field did not lose marking potency appreciably over time. The minimum number of days mice must consume toxic baits to achieve 100% lethality is 7 days for chlorophacinone, 3 days for bromethalin, brodifacoum, and bromadiolone (90%), but only 40% lethality was achieved for diphacinone/Ramik Green® during 7 day no-choice laboratory trials (Pitt et al. 2010). However, consumption of Ramik Green® in laboratory studies may have been influenced by the presence of the biomarker. The broadcast placebo baits used in this trial were available to wild house mice for the minimum 7-day toxicant bait exposure period.

Bait disappearance rates were directly proportional to estimated mouse densities even though some bait was probably consumed by other species including black rats (Figs. 5-7). Invertebrates may have impacted bait disappearance rates however their respective abundances were not sampled. Mongooses and feral cats were observed moving through the plots via the trail cameras. One mongoose was observed chewing but not consuming a Ramik® bait pellet; the single observation suggests that it is not likely that mongooses consumed much bait. Feral cats were not observed consuming baits. Several species of birds (nene, kolea, house finch, myna) were seen in the plots but none were observed to have sampled baits, although it would seem likely that birds with vegetative or generalist diets (sparrows, geese, etc…) would have been attracted to grain pellet baits (Witmer 2001). Feral pigs were observed in the plots on camera and in person but there were no actual observations of pigs eating placebo rodenticide baits scattered on the ground. Pigs captured via camera traps were moving through the baited plots and not foraging for baits. In a previous rodenticide broadcast trial on Hawaii Island feral pigs were documented to consume large quantities of fish-flavored Eaton rodenticide baits through raiding bait stations, but it was also likely that feral pigs foraged on the ground for broadcast baits (Pitt et al. 2006).

A very high proportion of mice within the sampling grids were marked (~96%) and unmarked mice were captured throughout the trapping grid, not just along the edges, thus no trapping edge-effects were observed, indicative of both small home range sizes (<20m) and low-levels of immigration (≤ 5%) into

the plots from adjacent habitats within the short 4-day trapping session. Unmarked mouse capture rates did not increase during the 4 night removal trapping period further illustrating either a lack of immigration into the plots or a large buffer of marked mice existed around the plots. Previous researchers have suggested that baiting can cause a temporary contraction of a more diffuse small mammal population towards bait, which would then re-disperse away from the baiting area when bait supply is exhausted, thus resulting in a buffer of marked rodents surrounding test plots (Chitty 1937, Smith 1971, Gurnell and Gipps 1989).

On several occasions trapping success within a particular plot was 27-31 mice per night with 49 traps set (~59% trap success). This would suggest there was a degree of trap saturation, that is to say that mice that otherwise could have been trappable did not locate open unoccupied traps (Nelson and Clarke 1973, Xia and Boonstra 1992, Beauvais and Buskirk 1999, Dunlevy et al. 2000). Thus our depletion-trapping density estimations probably underestimated actual population sizes within the plots. Additionally, four nights of trapping proved too small a sampling effort to effectively deplete the trapping grids of resident (marked) mice in this arid coastal study site, which further weaken our population estimates. However, for our purposes, the trapping effort was sufficient to conclude that all resident mice within the test plots were likely exposed to (and consumed) the two placebo bait formulations during the simulated rodenticide application.

The next portion of this study will compare and contrast the efficacy of broadcast toxic Ramik® bait (diphacinone (0.005%) and Rozol® (chlorophacinone (0.005%) rodenticides on mice in coastal Hawaiian field sites using these broadcast techniques and application rates. Assessing the efficacy of toxic baits on non-communal house mice, and non-target consumers, in field conditions is a required step before employing specific rodenticides as tools to eradicate introduced mice from sensitive ecological areas or small island habitats.

Acknowledgments:

This field research was funded by the U.S. Fish & Wildlife Service's Pacific Islands Office, 300 Ala Moana Boulevard, Room 3-122, Honolulu, HI 96850. The study was approved as NWRC Protocol QA-1857. We are grateful to Catherine Swift (USFWS) for her continuing support of invasive rodent control and habitat conservation efforts in the Pacific region; to the respective bait manufacturers that provided the placebo biomarker-treated bait formulations; and to Aaron Shiels for help in the field. Rhonda Loh of Hawaii Volcanoes National Park helped facilitate permitting, access and logistics in the study site.

Figures:

Figure 1. HAVO coastal plains study site topographic map

Figure 2. Aerial photo of HAVO coastal plains study site (Treatment plots marked in red)

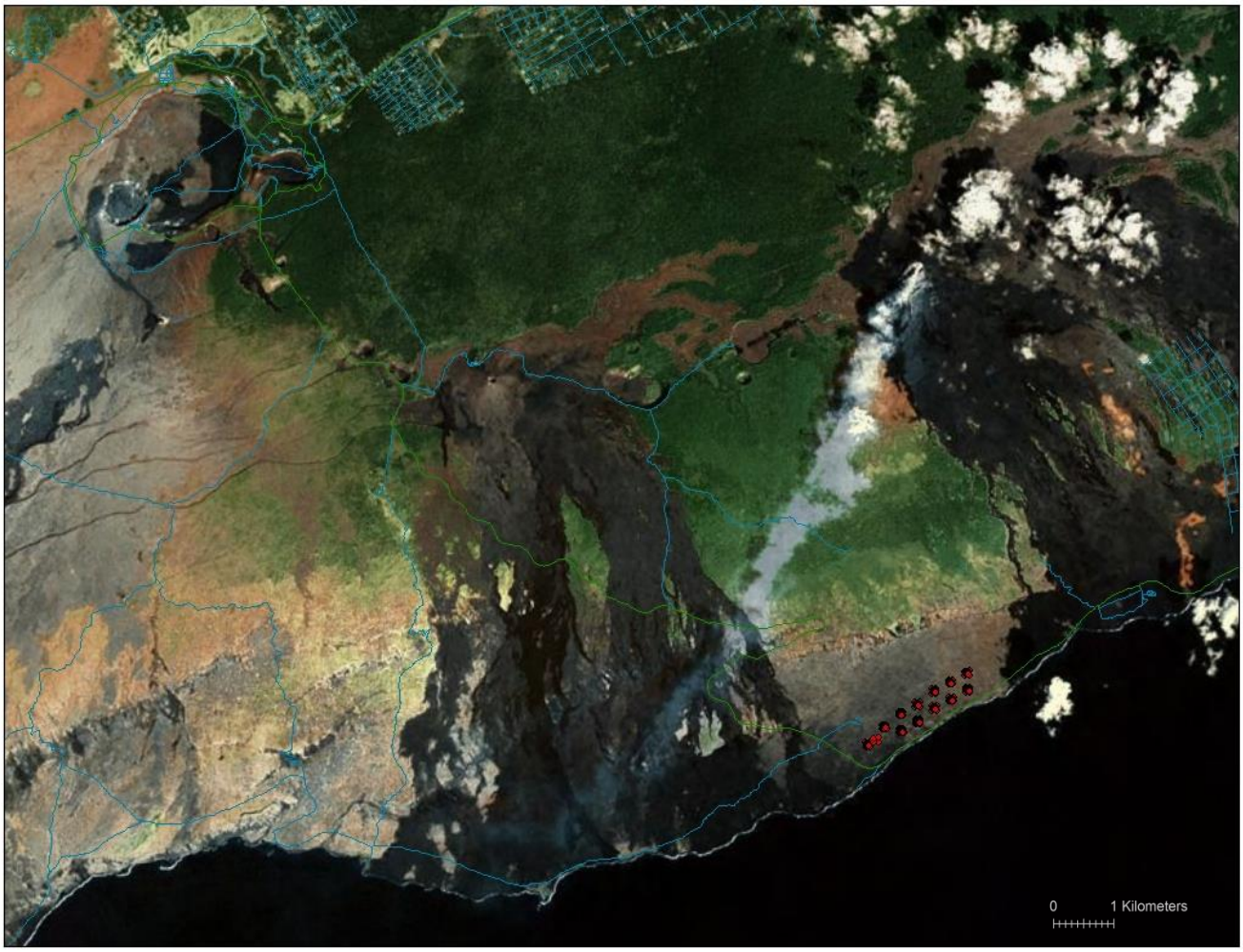


Figure 3. Locations (corners) of treatment plots (12) and plot trapping grid layout

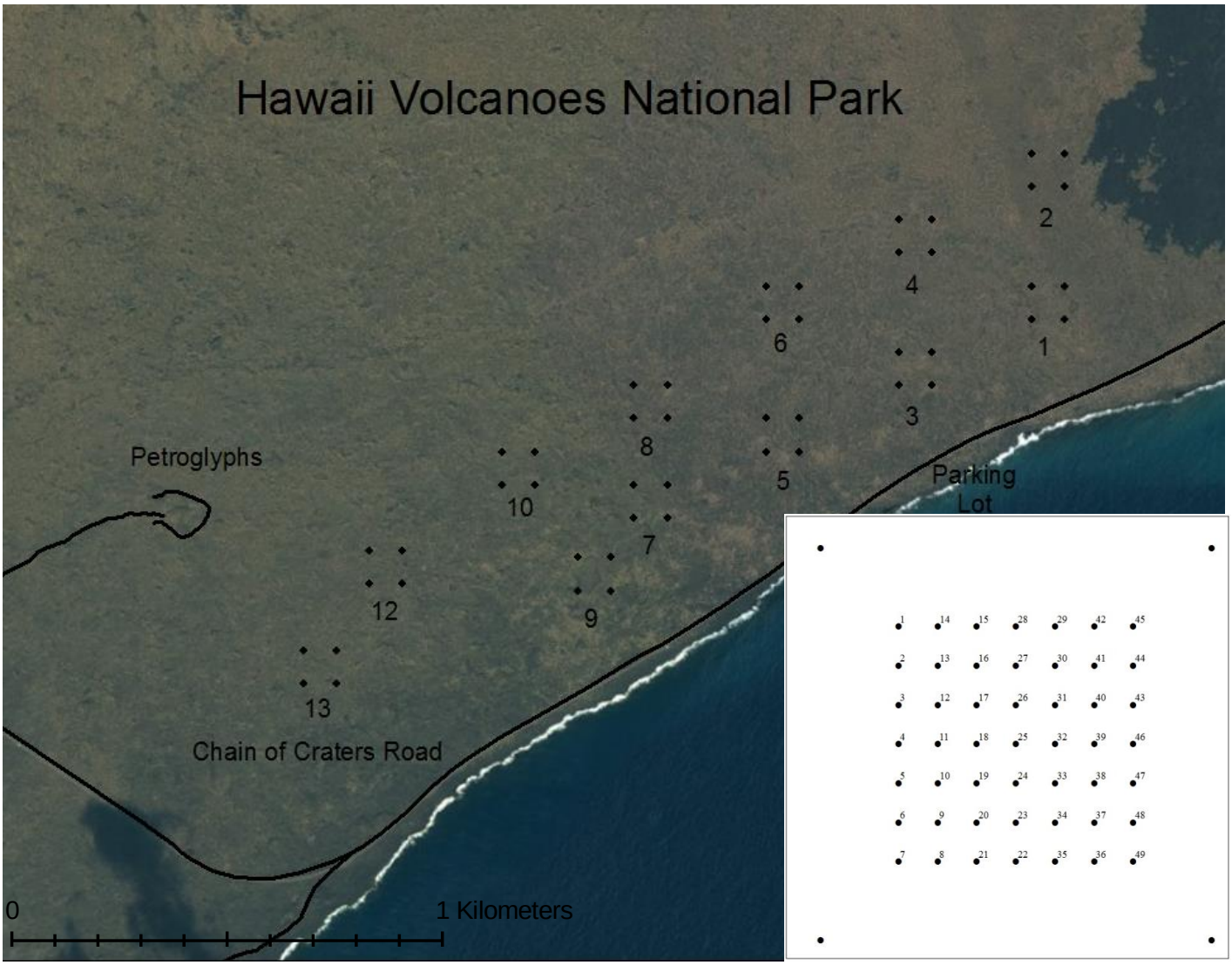


Figure 4. Daily rainfall totals for the entire study period.

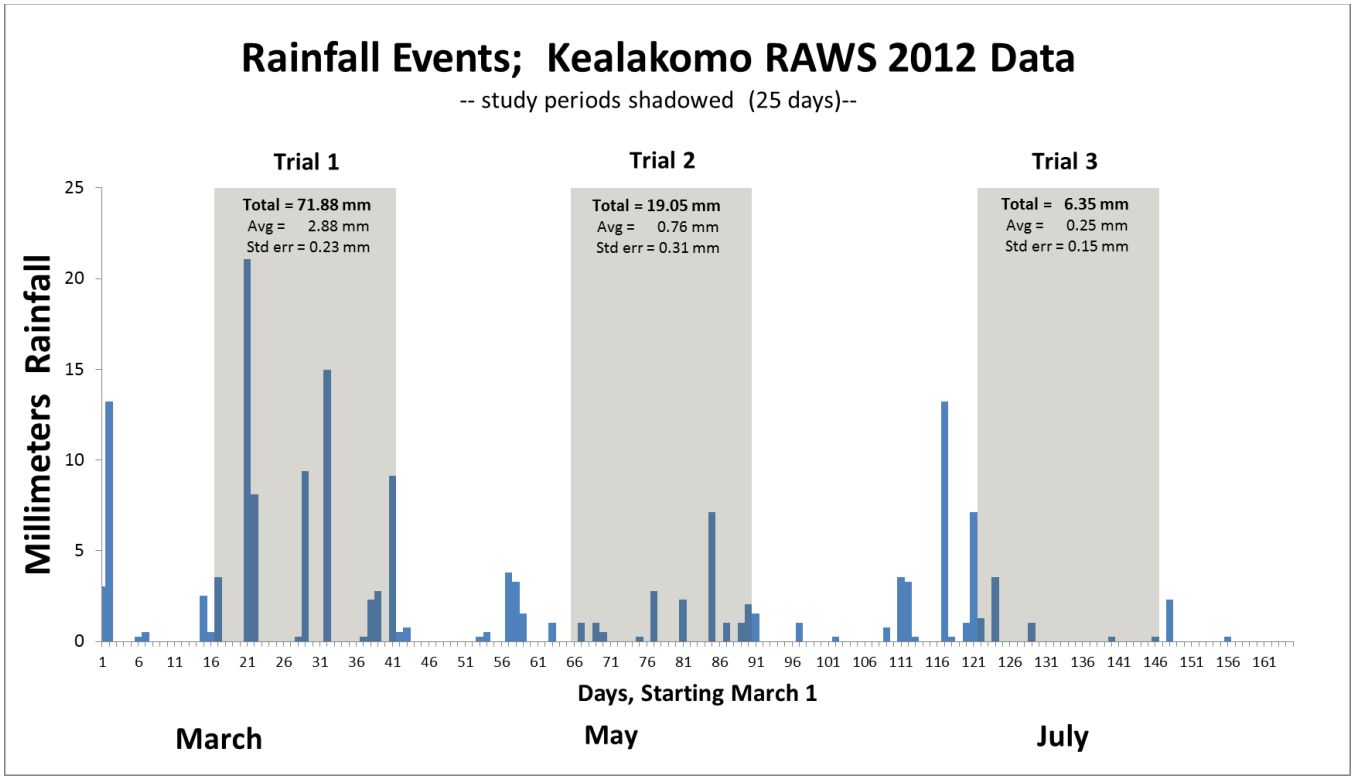
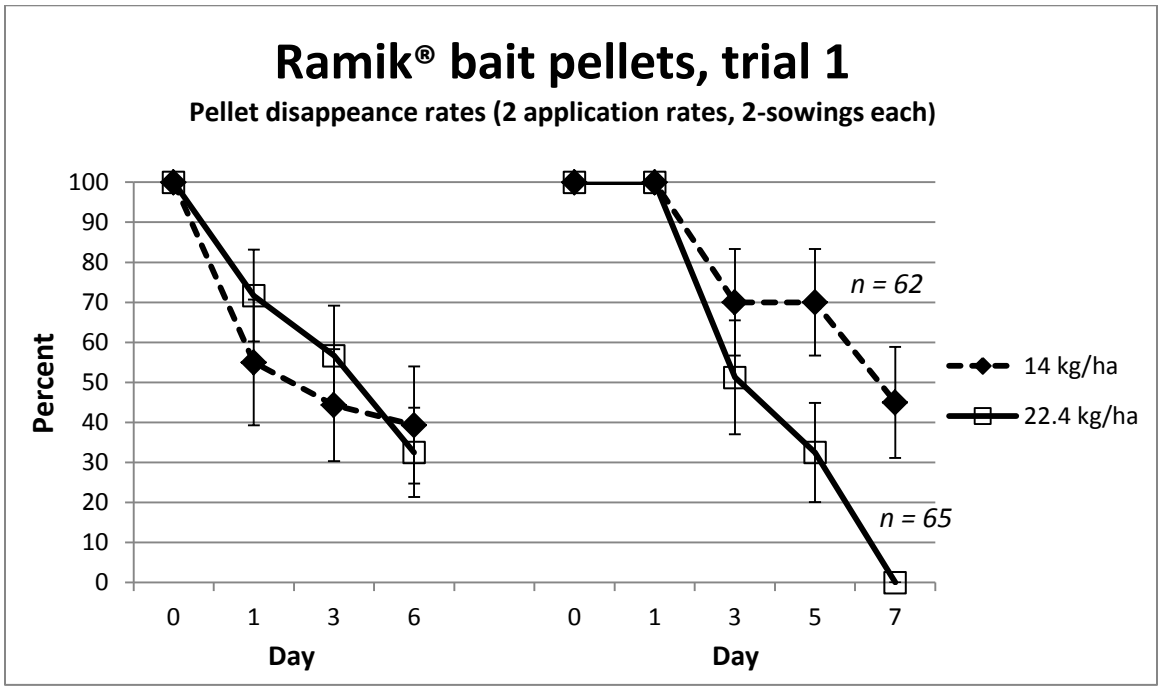


Figure 5. Bait disappearance rate by bait type, broadcast application rate, and exposure day- Trial 1. N is the number of mice captured in each treatment plot post-treatment.



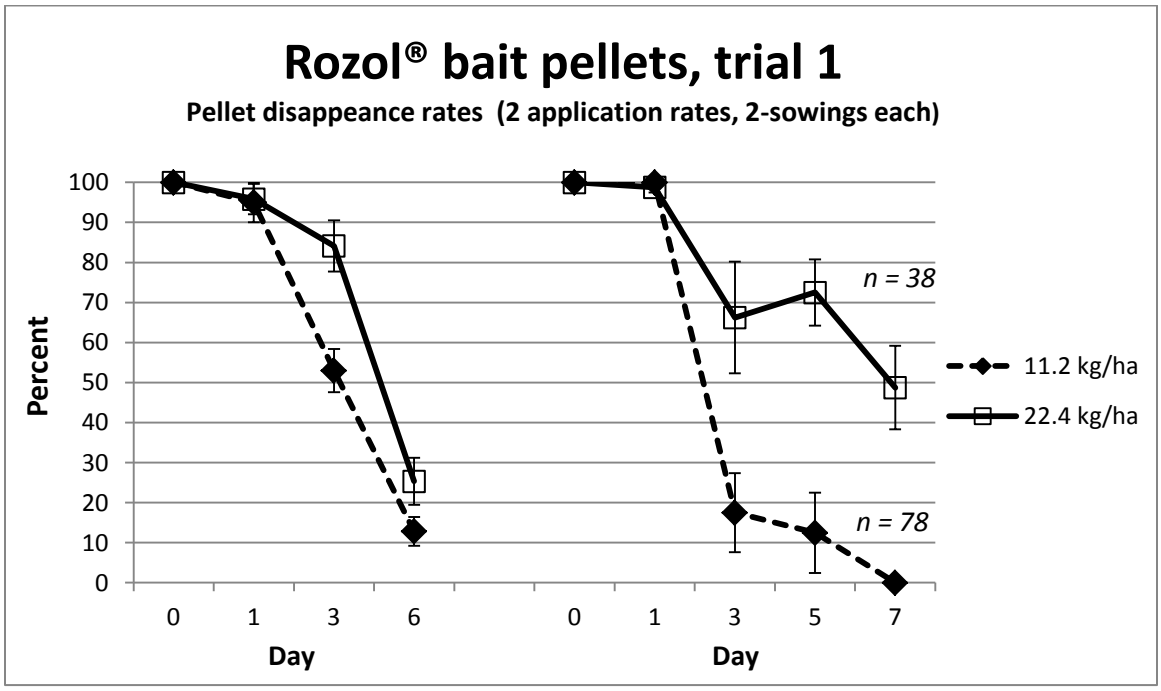
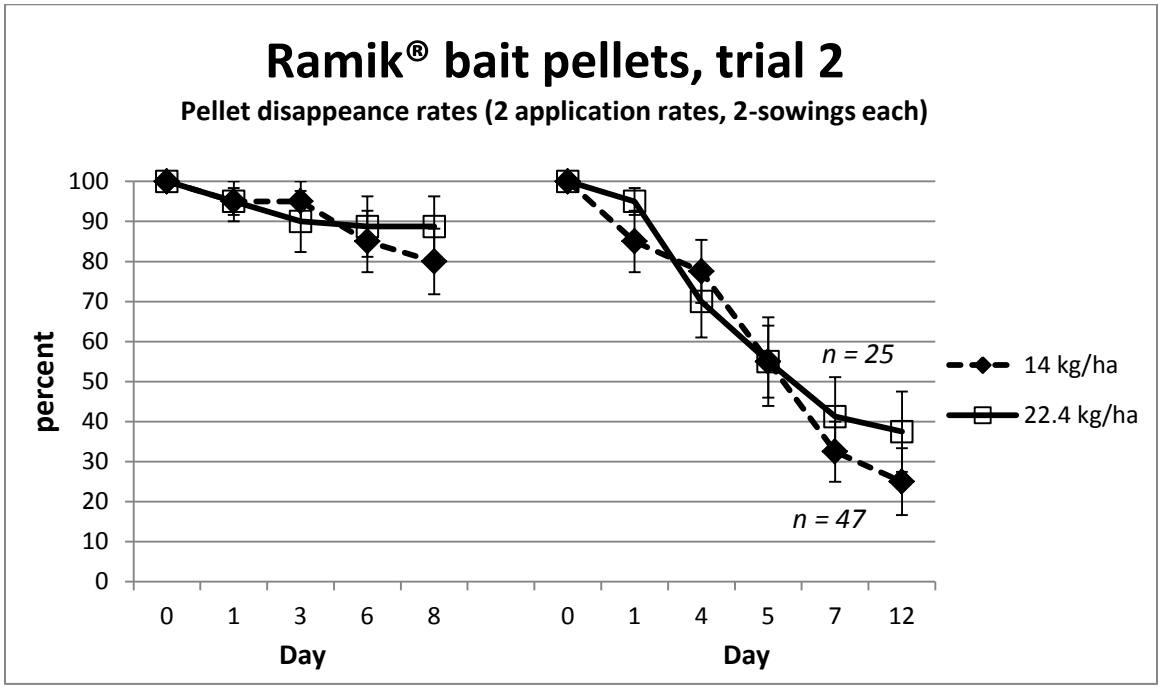


Figure 6. Bait disappearance rate by bait type, broadcast application rate, and exposure day- Trial 2. N is the number of mice captured in each treatment plot post-treatment.



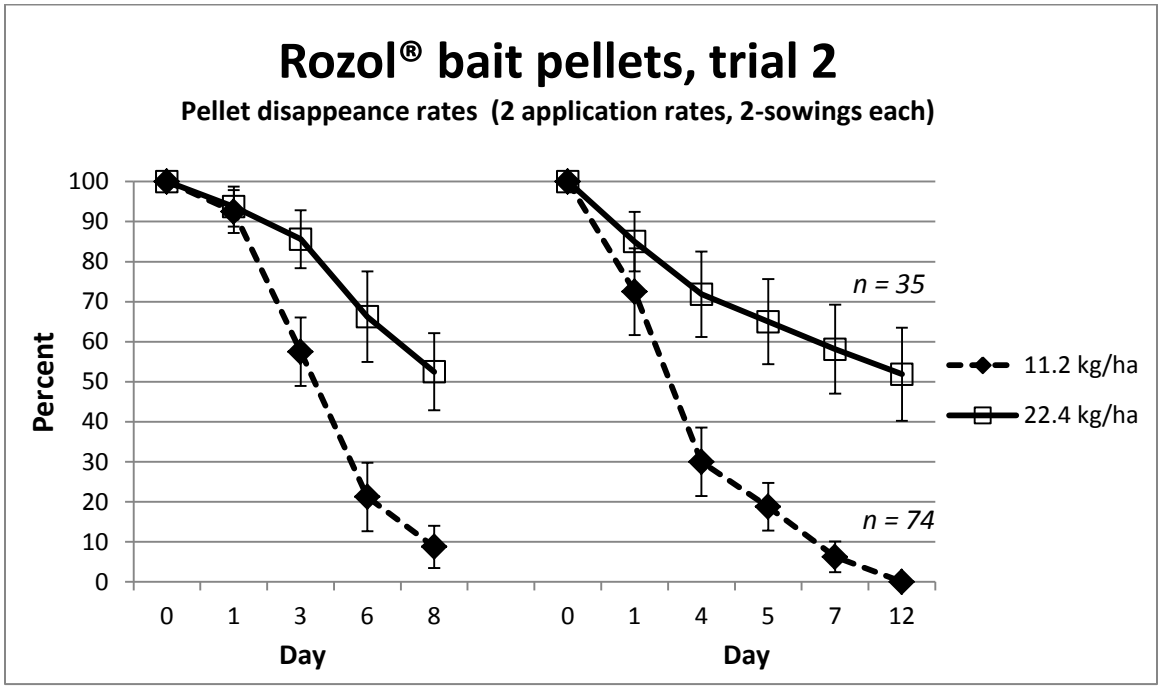
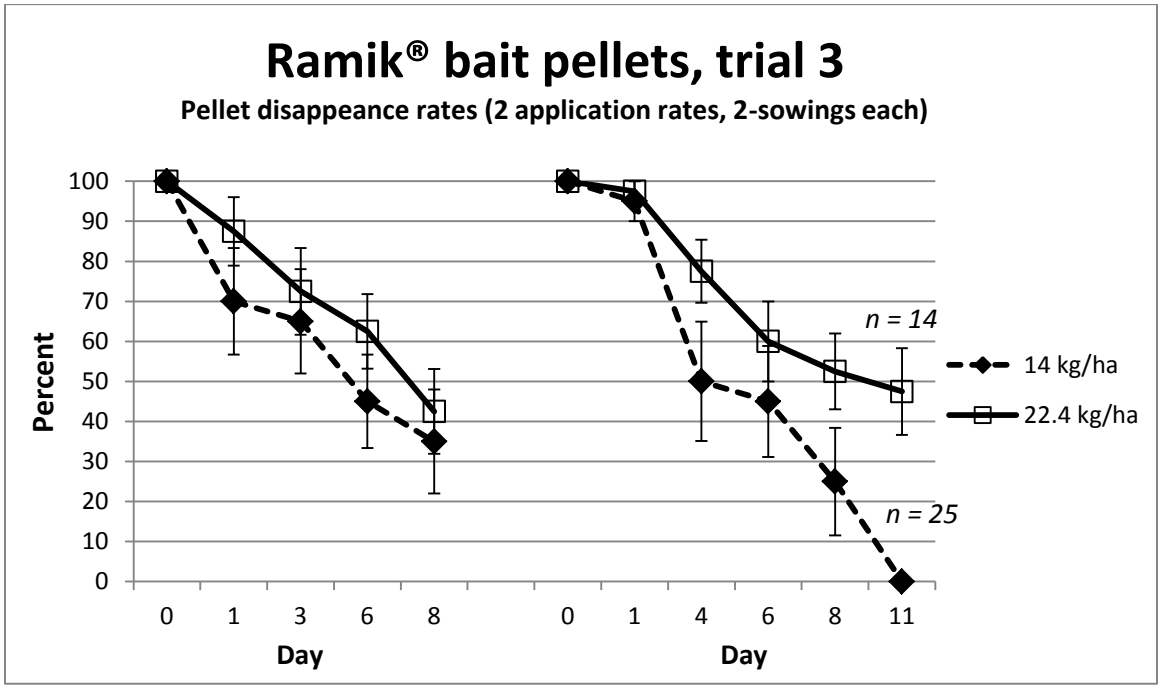


Figure 7. Bait disappearance rate by bait type, broadcast application rate, and exposure day- Trial 3. N is the number of mice captured in each treatment plot post-treatment.



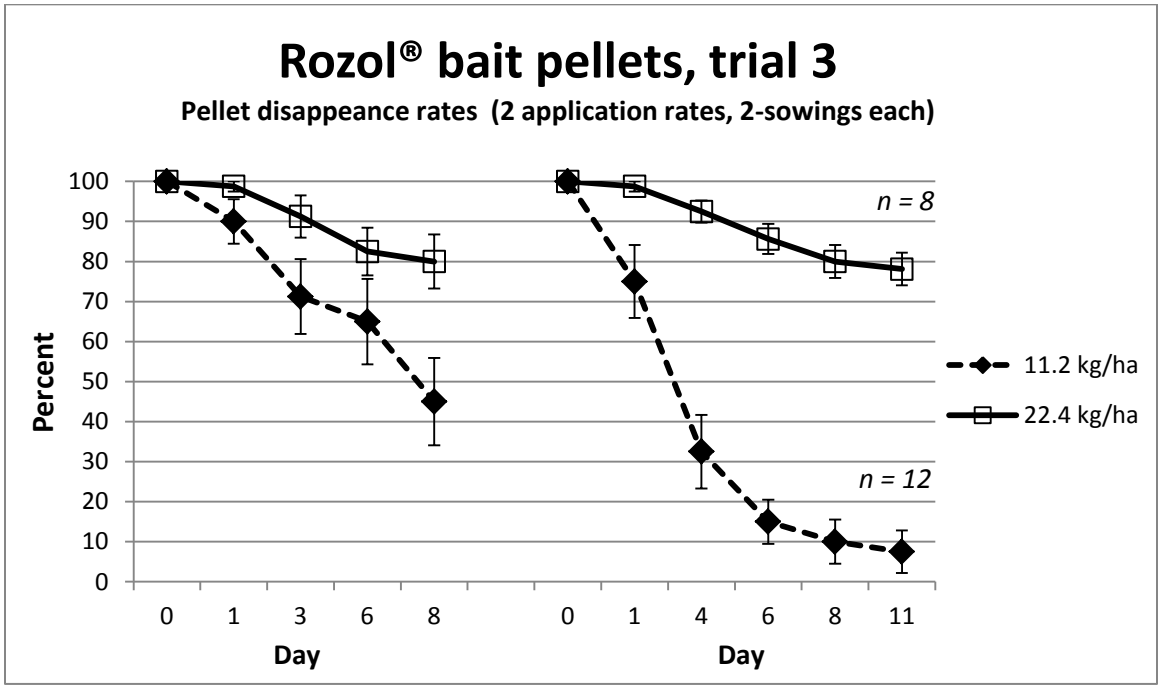


Figure 8. Results of depletion sampling within 60m by 60m trapping grids (12 plots) containing 49 traps each. Linear regression trend lines suggest that house mouse abundance ranged from about 150 to 300 or more mice per hectare. 95% of captures were marked suggesting very low immigration rates.

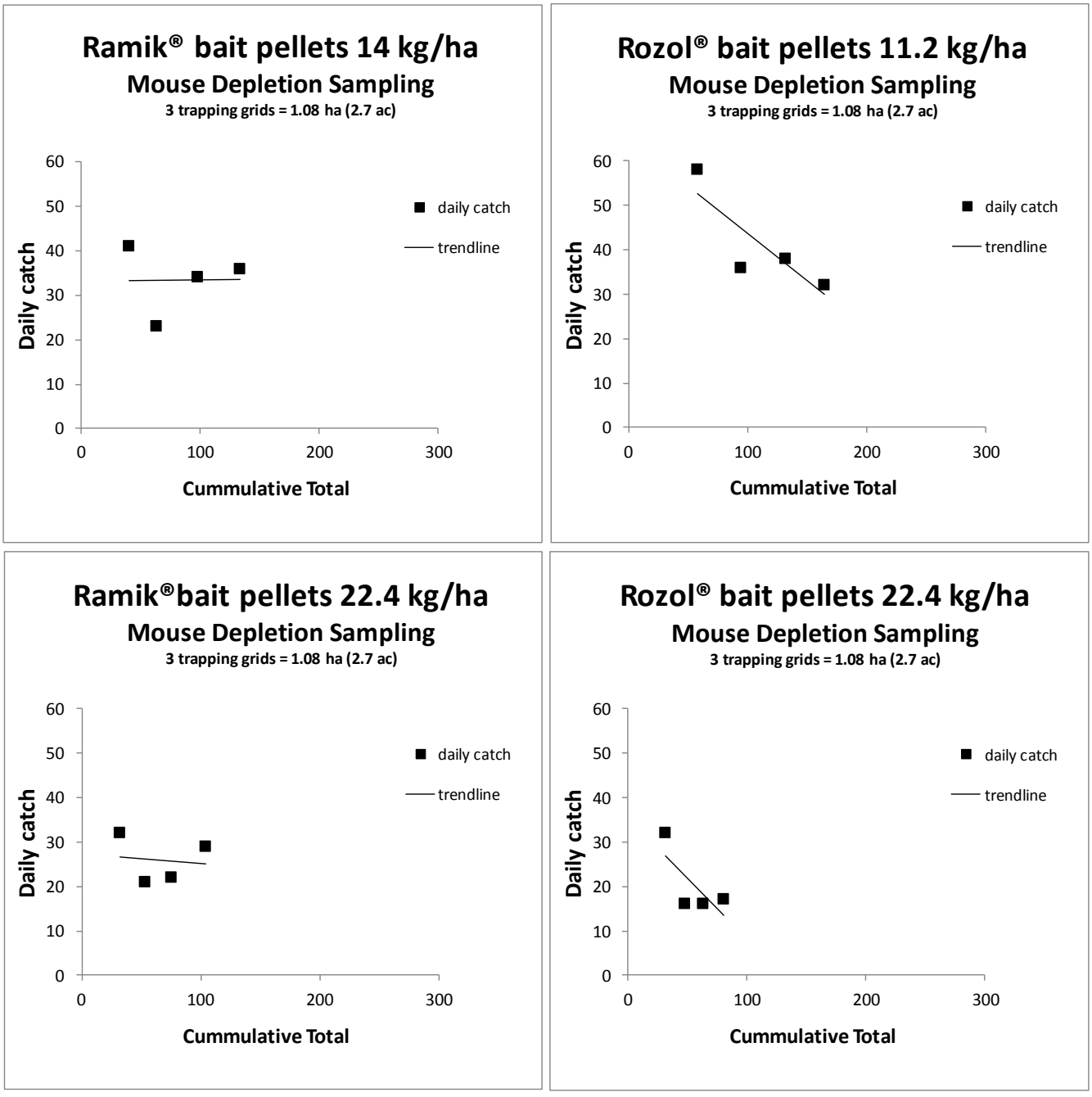


Figure 9a. Percentage of mice marked by baiting regime and trapping day- Trial 1.

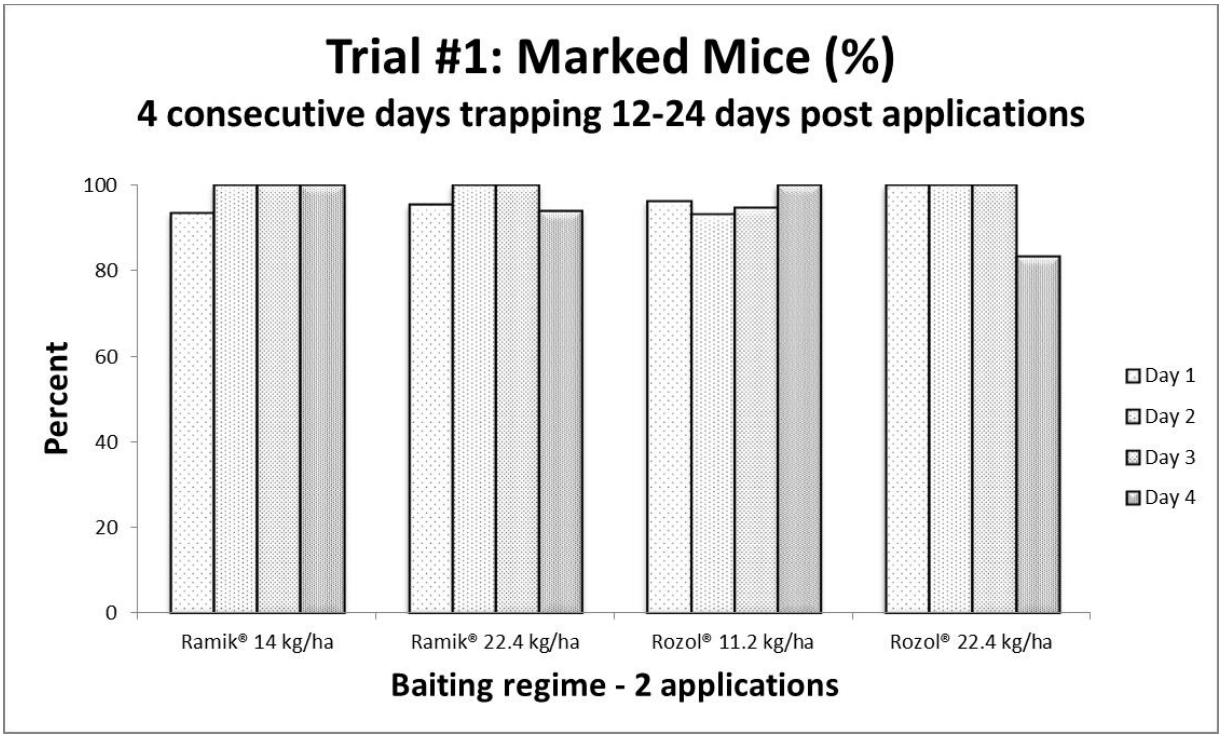


Figure 9b. Percentage of mice marked by baiting regime and biomarker detection class- Trial 1.

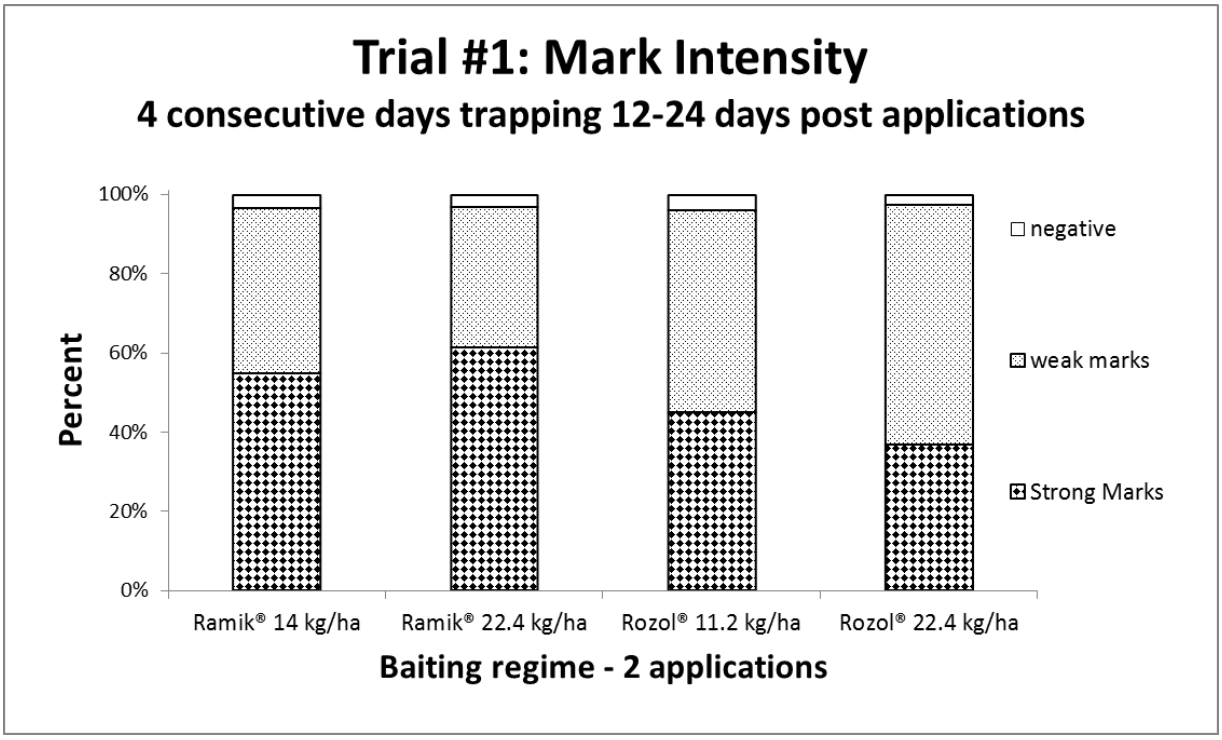


Figure 10a. Percentage of mice marked by baiting regime and trapping day- Trial 2.

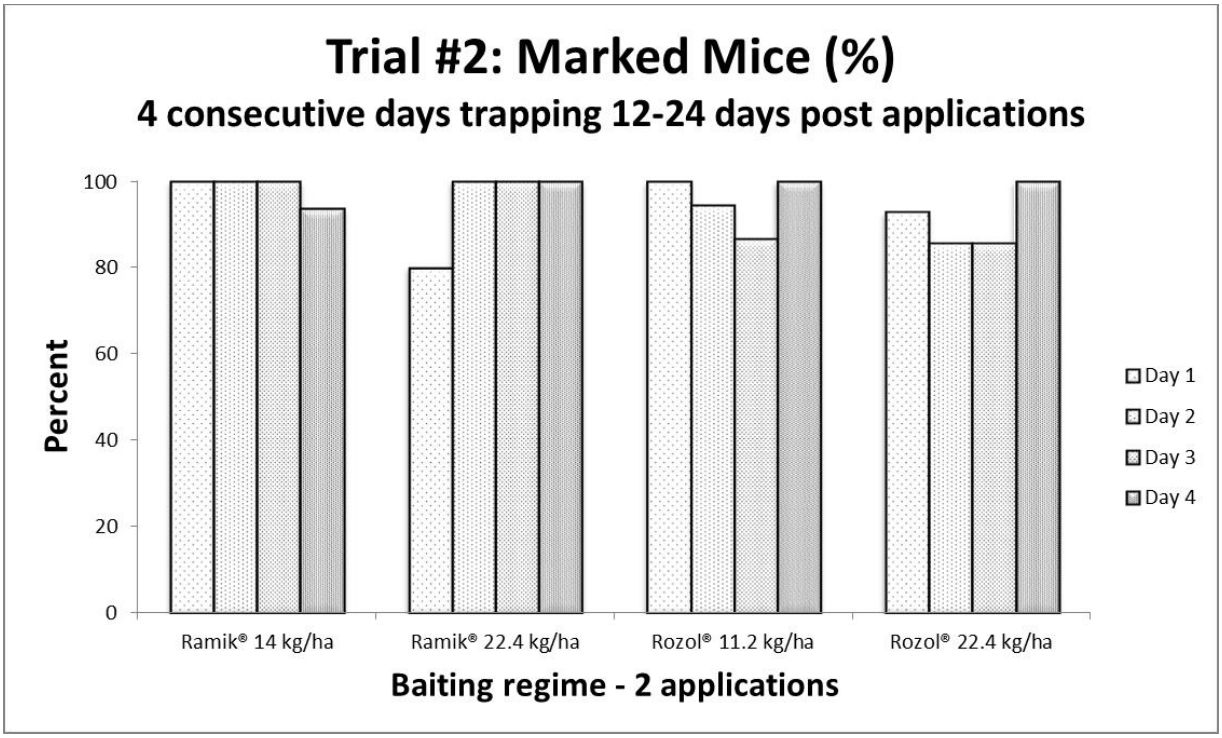


Figure 10b. Percentage of mice marked by baiting regime and biomarker detection class- Trial 2.

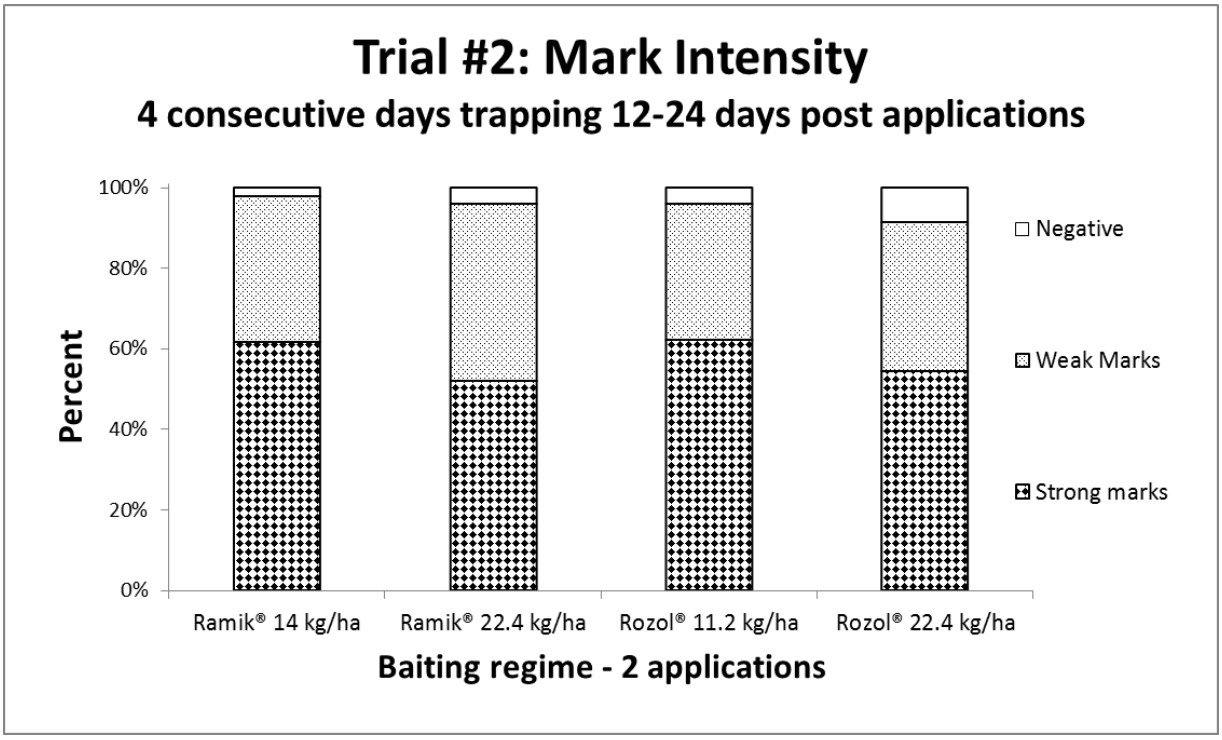


Figure 11a. Percentage of mice marked by baiting regime and trapping day- Trial 3.

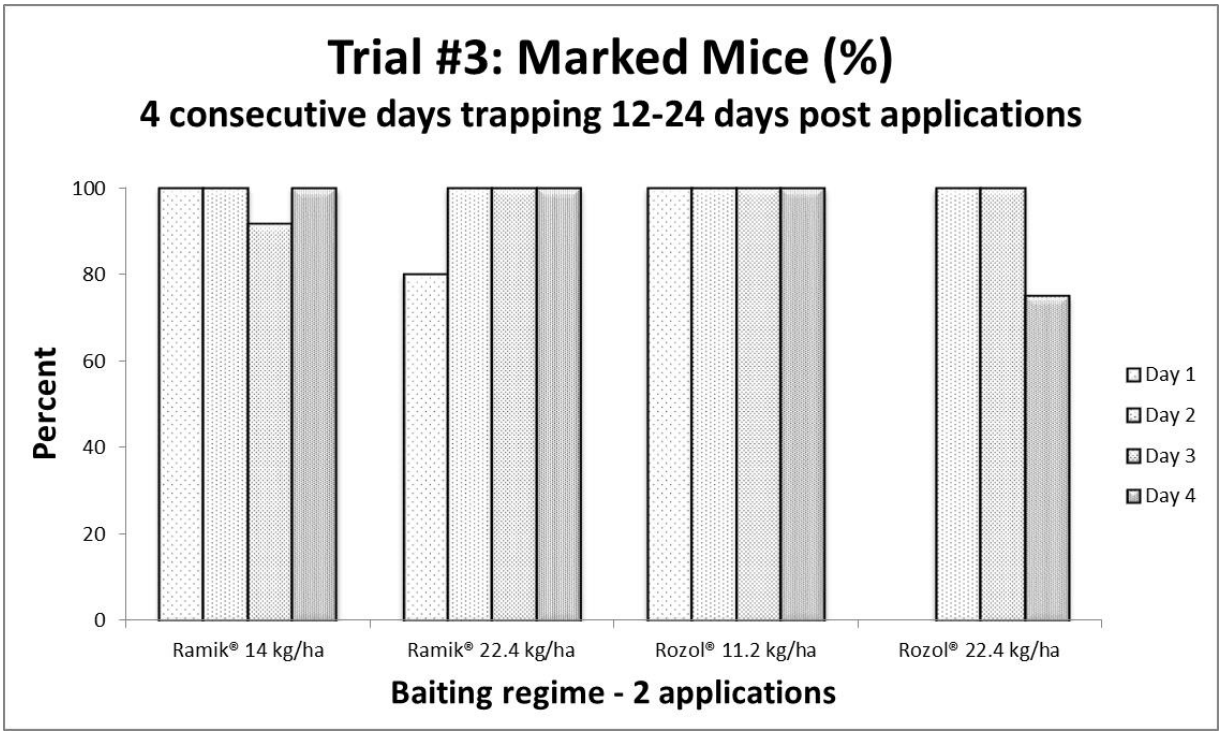


Figure 11b. Percentage of mice marked by baiting regime and biomarker detection class- Trial 3.

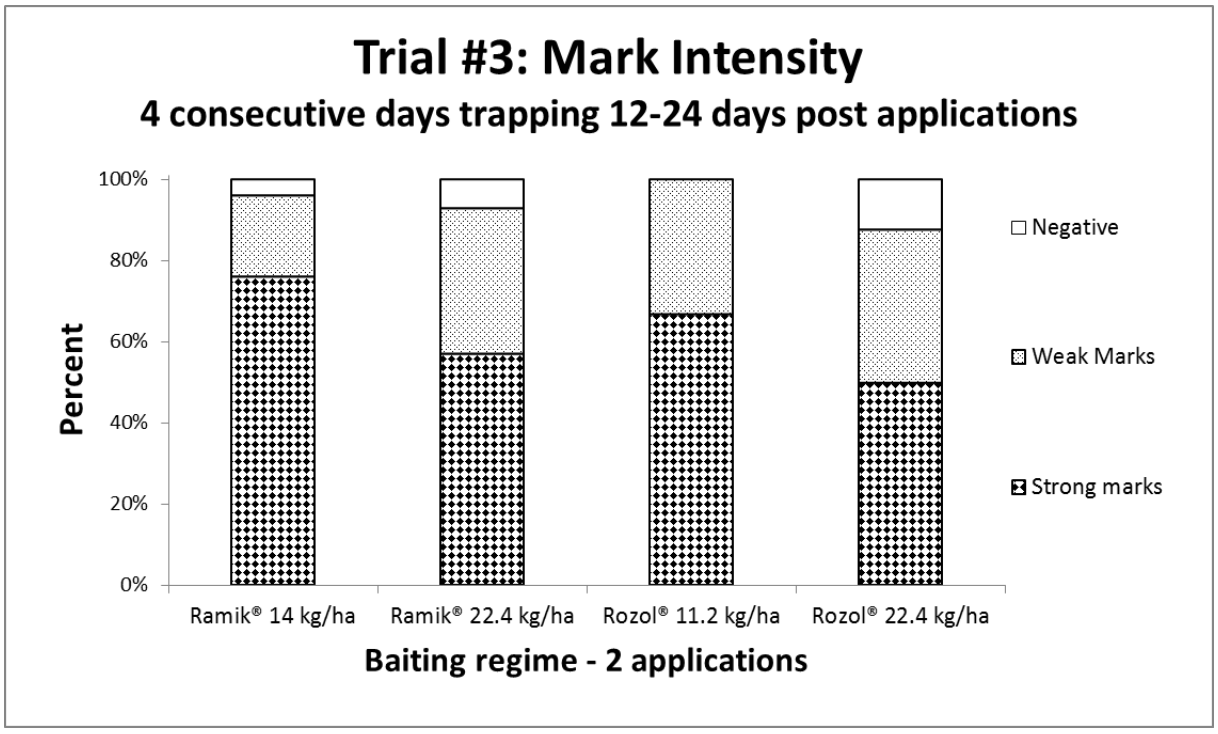


Figure 12. Mean percentage mice marked for all trials by bait type, application rate and trapping day

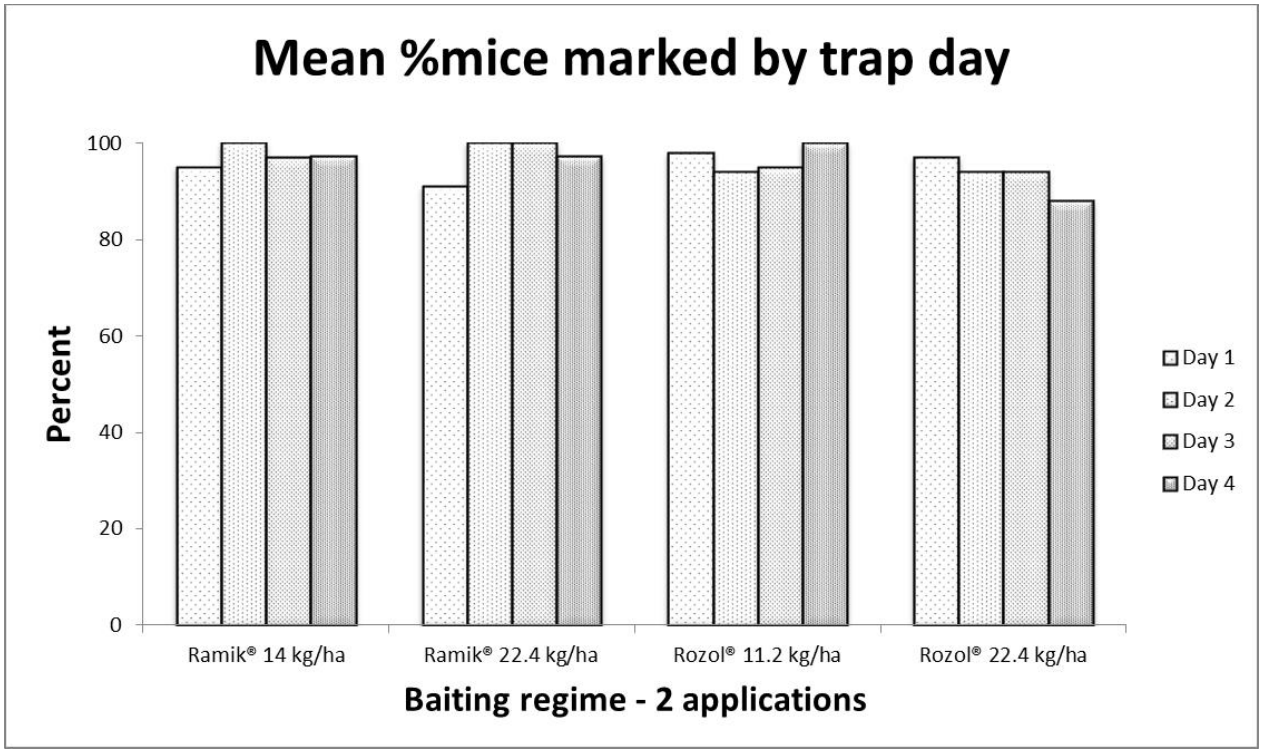


Figure 13. Spatial distribution of mice captures and biomarker marking intensity
Treatment- Ramik® bait pellets14 kg/ha.

Legend: Tiny black dots = no catch, small colored dots = weak markings, large colored dots = strong markings, bright red dots = unmarked, m = male, f = female

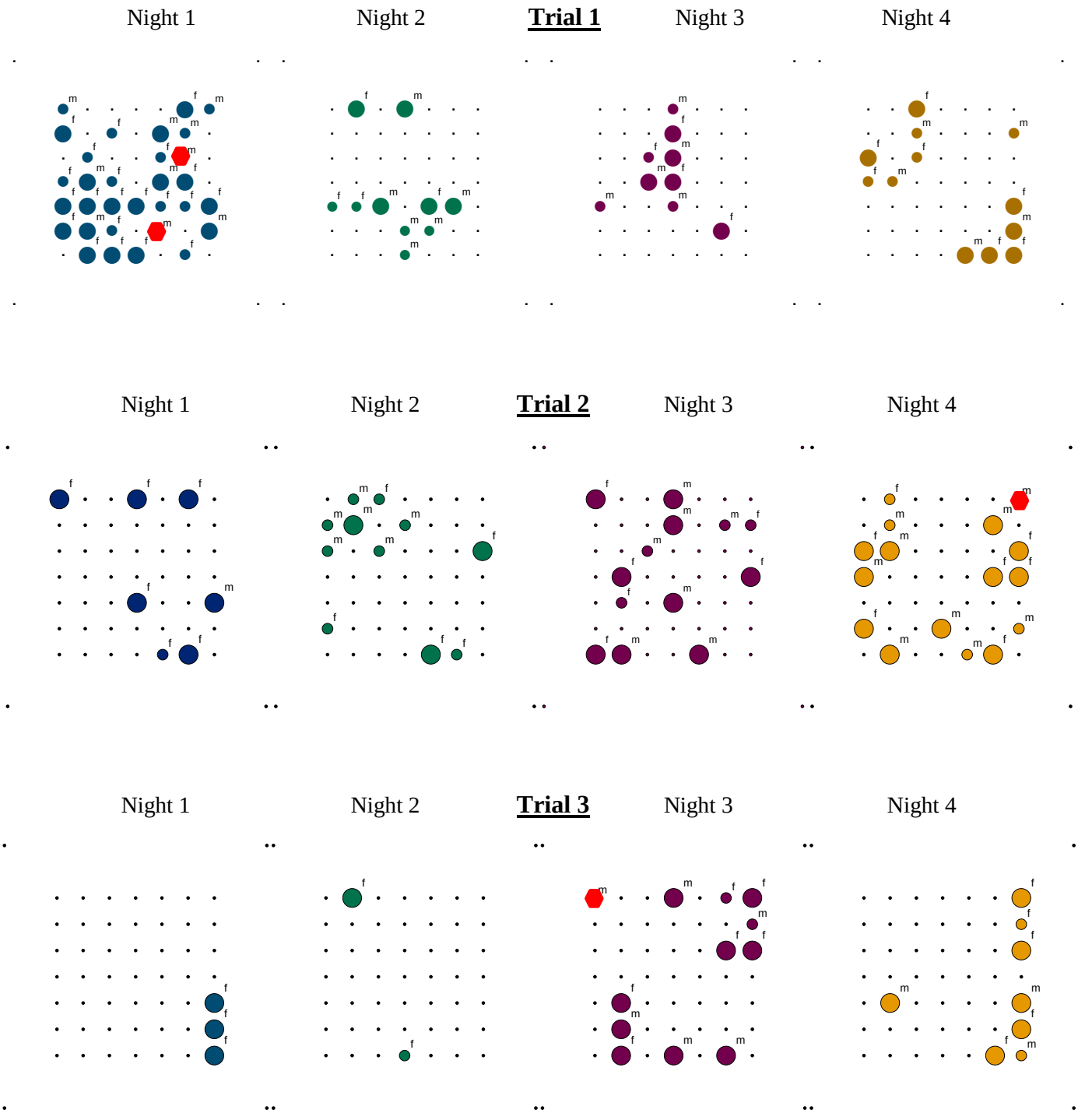


Figure 14. Spatial distribution of mice captures and biomarker marking intensity
Treatment- Ramik® bait pellets 22.4 kg/ha.

Legend: Tiny black dots = no catch, small colored dots = weak markings, large colored dots = strong markings, bright red dots = unmarked, m = male, f = female

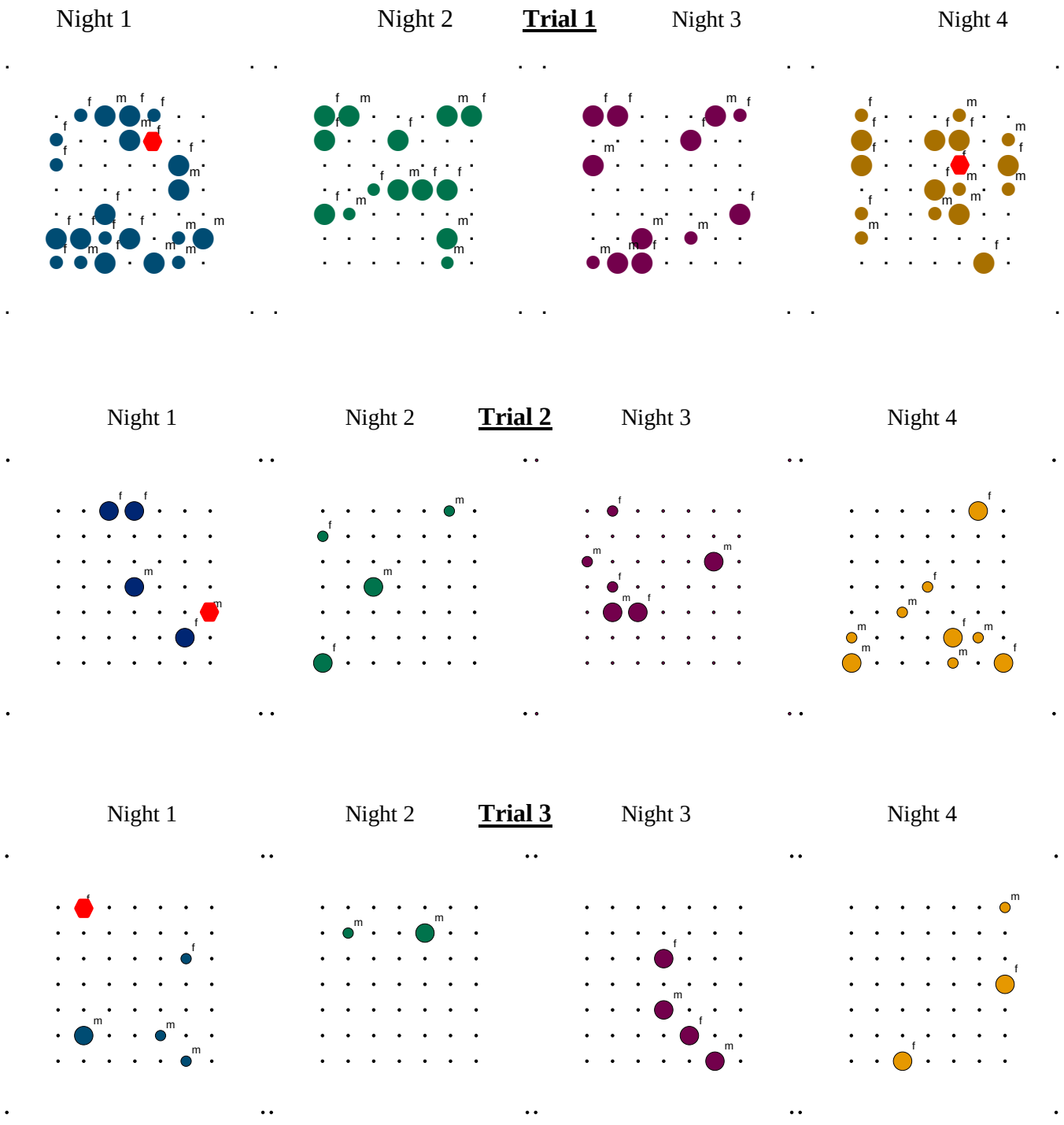


Figure 15. Spatial distribution of mice captures and biomarker marking intensity Treatment- Rozol® bait pellets 11.2 kg/ha.

Legend: Tiny black dots = no catch, small colored dots = weak markings, large colored dots = strong markings, bright red dots = unmarked, m = male, f = female

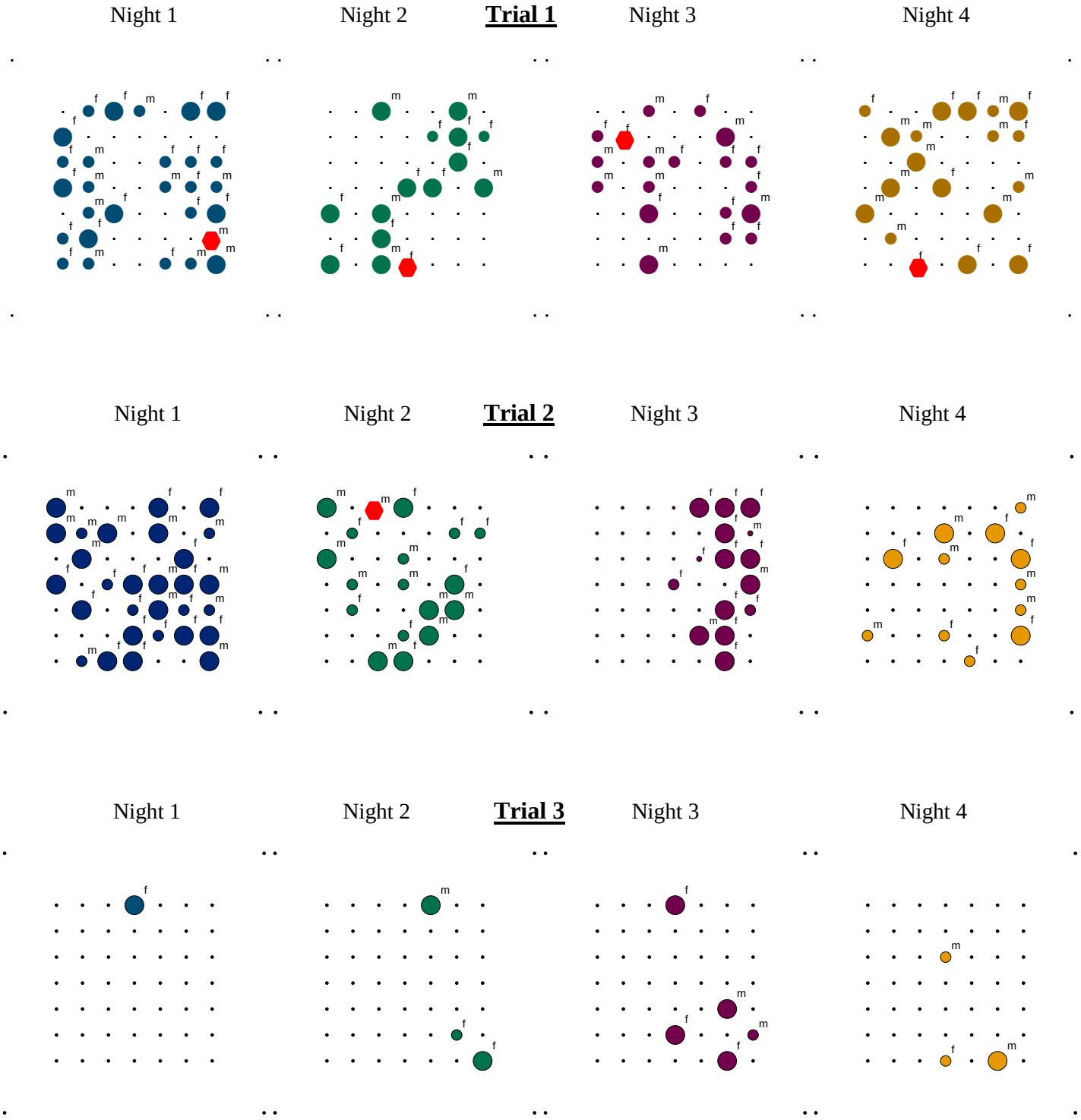


Figure 16. Spatial distribution of mice captures and biomarker marking intensity
Treatment- Rozol® bait pellets 22.4 kg/ha.

Legend: Tiny black dots = no catch, small colored dots = weak markings, large colored dots = strong markings, bright red dots = unmarked, m = male, f = female

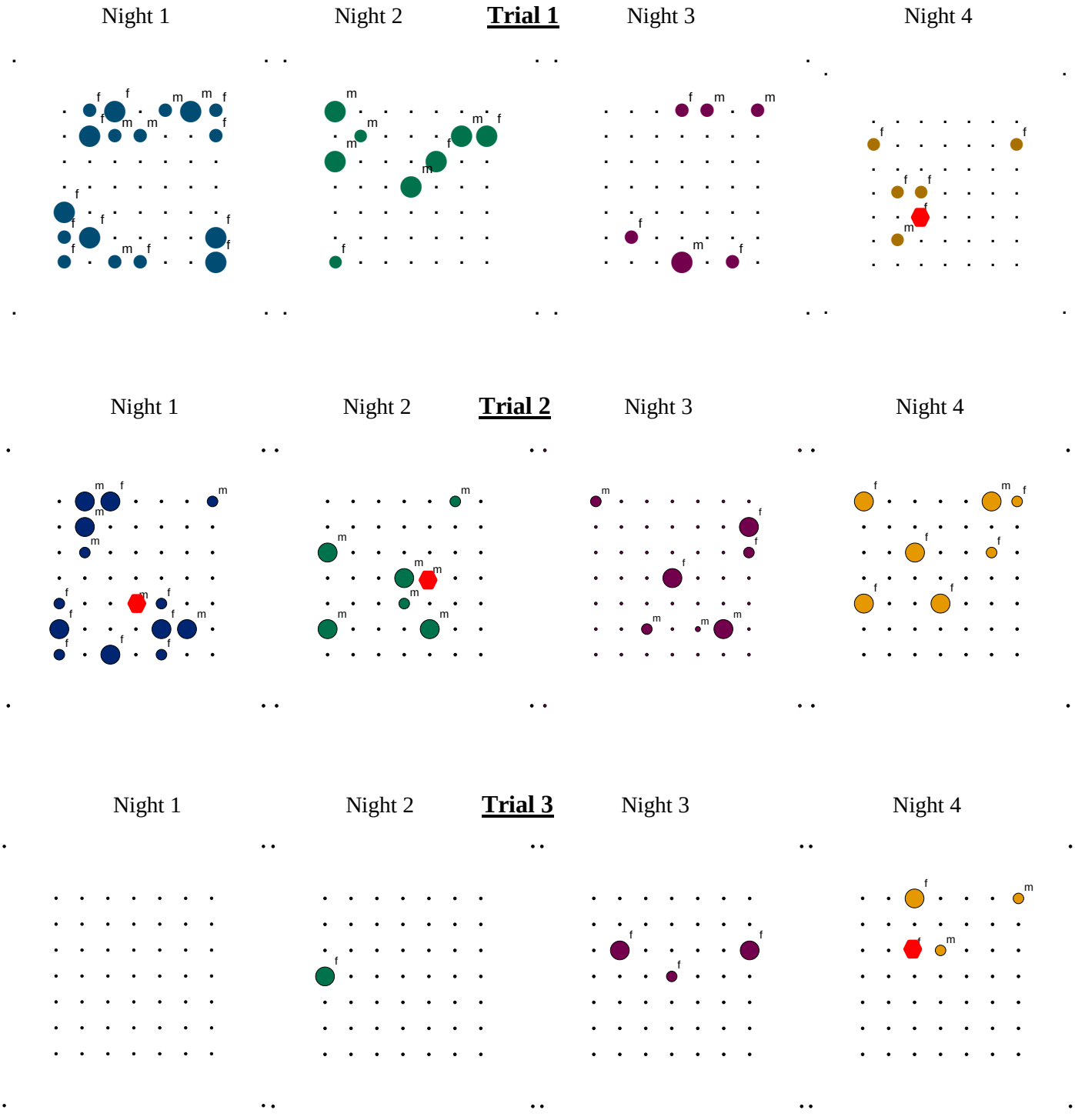


Figure 17. Mouse weight distributions by gender and reproductive (pregnant females- palpitation).

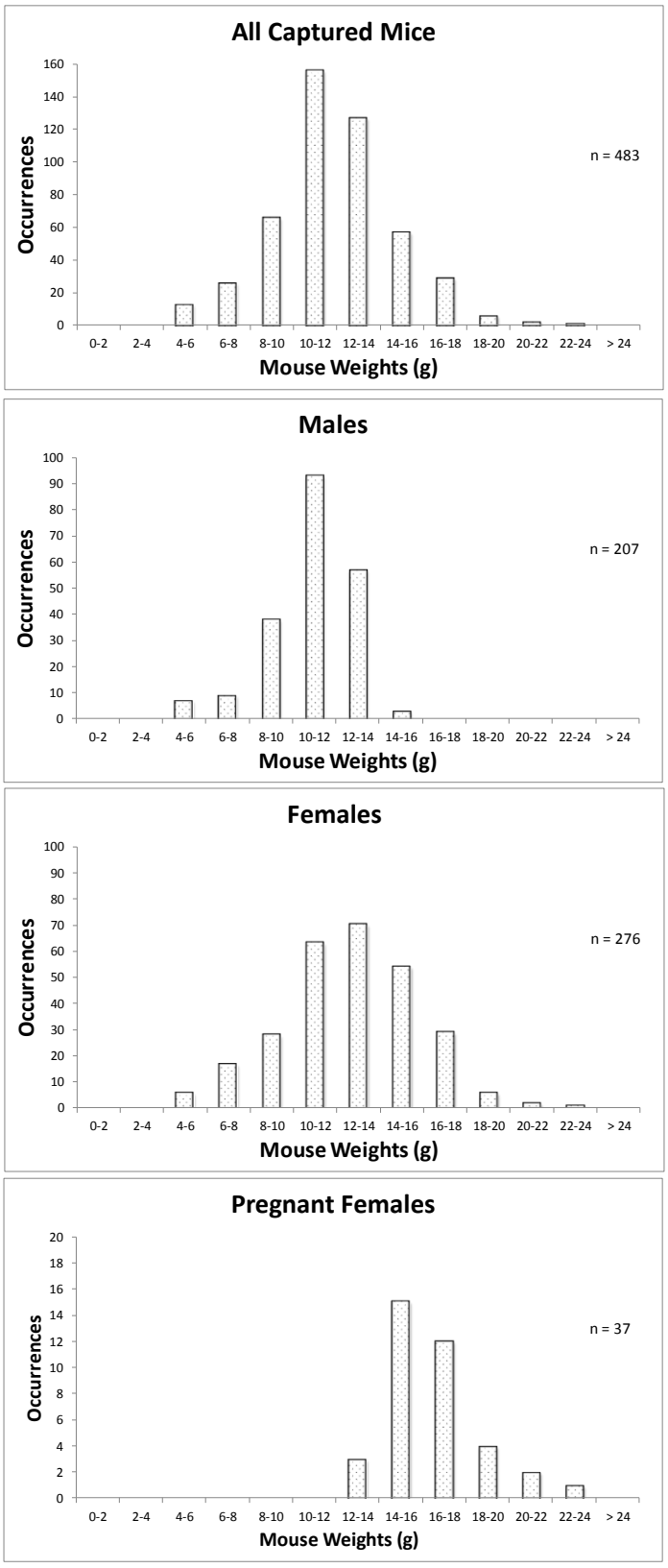


Figure 18. Mouse weight distribution by Trial period

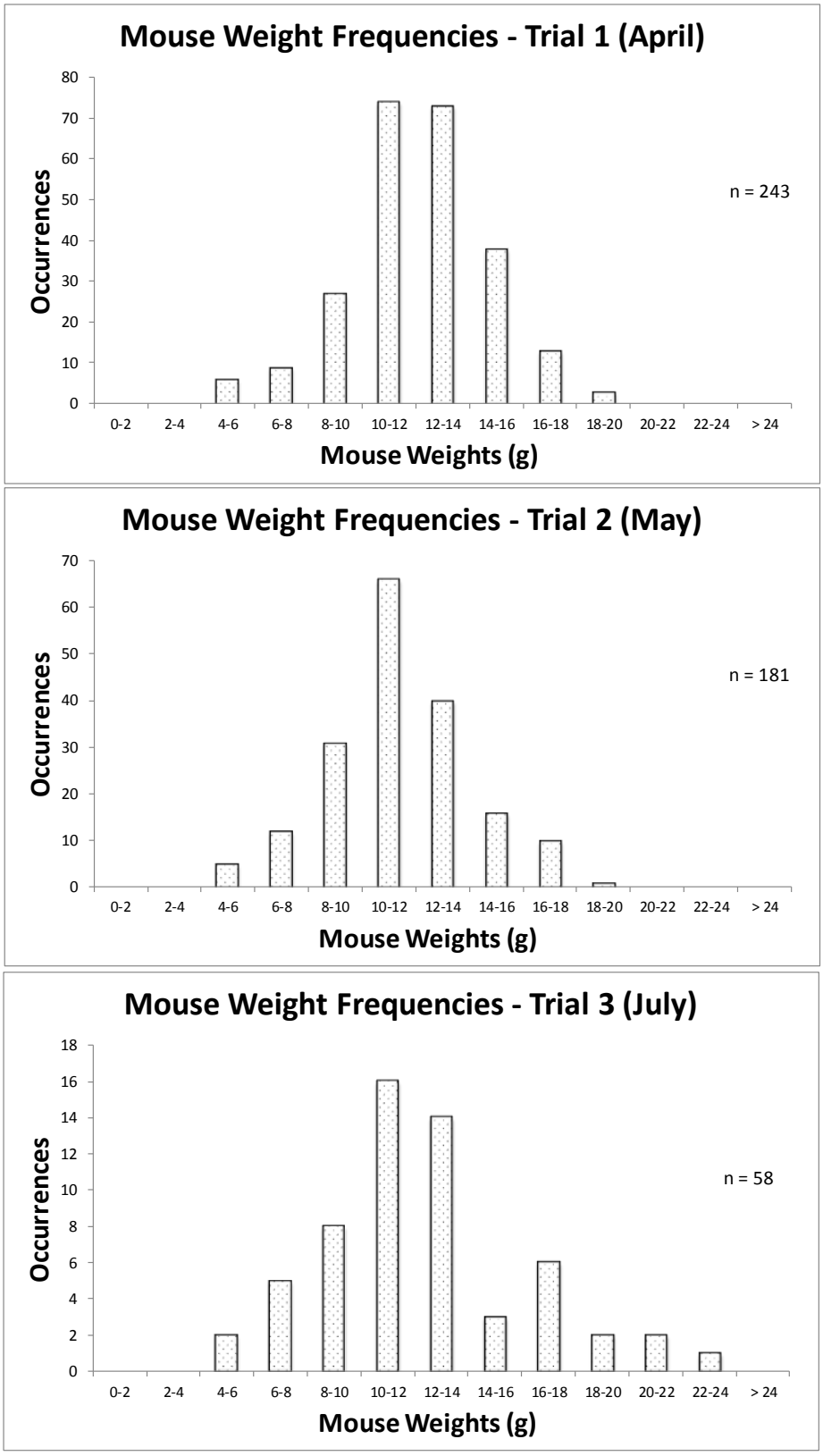


Table 1. Number of mice captured and DMCT marking by baiting regime and trapping day.

				# Mice captured (# marked) by trapping day				
Bait type	**Application rate**	**Trial**	**Plot #**	**1**	**2**	**3**	**4**	**1-4**
Ramik® Bait Pellets	14 kg/ha	1	2	31(29)	10(10)	9(9)	12(12)	62(60)
		2	8	7(7)	11(11)	13(13)	16(15)	47(46)
		3	9	3(3)	2(2)	12(11)	8(8)	25(24)
			All plots	41(39)	23(23)	34(33)	36(35)	134(130)
	22.4 kg/ha	1	1	22(21)	14(14)	12(12)	17(16)	65(63)
		2	10	5(4)	5(5)	6(6)	9(9)	25(24)
		3	13	5(4)	2(2)	4(4)	3(3)	14(13)
			All plots	32(29)	21(21)	22(22)	29(28)	104(100)
Rozol® Bait Pellets	11.2 kg/ha	1	3	28(27)	15(14)	18(18)	17(17)	78(76)
		2	6	29(29)	18(17)	15(13)	12(12)	74(71)
		3	7	1(1)	3(3)	5(5)	3(3)	12(12)
			All plots	58(57)	36(34)	38(36)	32(32)	164(159)
	22.4 kg/ha	1	4	18(18)	8(8)	6(6)	6(5)	38(37)
		2	5	14(13)	7(6)	7(6)	7(7)	35(32)
		3	12	0(0)	1(1)	3(3)	4(3)	8(7)
			All plots	32(31)	16(15)	16(15)	17(15)	81(76)

Table 2. Average house mouse body weights by treatment and plot.

Treatment	# mice	avg weight (g)	standard error
Ramik® 14.4	134	12.95	0.24
Ramik® 22.4	104	11.90	0.27
Rozol® 11.2	164	11.44	0.22
Rozol® 22.4	81	11.53	0.31

Plot #	# mice	avg weight (g)	standard error
1	65	12.34	0.34
2	62	12.89	0.35
3	78	11.86	0.31
4	38	12.03	0.44
5	35	11.25	0.46
6	74	10.94	0.32
7	12	11.82	0.79
8	47	12.33	0.40
9	25	14.28	0.55
10	25	11.66	0.55
12	8	10.33	0.97
13	14	10.29	0.72

Table 3. Pooled average house mouse body weights and sex ratios by treatment.

		mice						rats	
Plot #'s	Treatment kg/ha	total	m	f	avg (g)	min (g)	max (g)	total	avg (g)
2,8,9	Ramik® 14.4	134	58	76	12.95	5.5	23.6	0	
1,10,13	Ramik® 22.4	104	47	57	11.90	4.2	20.4	0	
3,6,7	Rozol® 11.2	164	68	96	11.44	4.2	17.9	3	29.6
4,5,12	Rozol® 22.4	81	34	47	11.53	5.1	17.9	0	
	Totals	483	207	276	12.0	4.2	23.6	3	29.6

		mice						rats	
Plot #'s	night	total	m	f	avg (g)	min (g)	max (g)	total	avg (g)
all	1	163	58	105	12.1	4.2	19.4		
	2	96	50	46	11.9	4.7	23.6		
	3	110	48	62	11.9	4.2	20.4	1	16.2
	4	114	51	63	11.9	4.4	21.3	2	36.3

Table 4. DMCT fluorescence intensity by tissue type. The "internal" category represents mice that were negative upon external inspection but were later found to be positive (weak category) for DMCT after pulling incisors and observing bases under UV.

Marked Mice - Totals

All replicates (pooled)

			incisors					
date	Plot #'s	kg bait/ha	strong	weak	internal weak	neg	marked bones	total mice
April - July 2012	2,5,9	Ramik® 14.4	82	48	5	4	59	134
April - July 2012	1,6,13	Ramik® 22.4	61	39	8	4	35	104
April - July 2012	3,8,7	Rozol® 11.2	89	69	12	6	60	164
April - July 2012	4,10,12	Rozol® 22.4	37	39	8	5	22	81

Marked Mice - Percentages

All replicates (pooled)

			incisors					
date	Plot #'s	bait	strong	weak	internal weak	neg	marked bones	total mice
April - July 2012	2,5,9	Ramik® 14.4	61	36	4	3	44	100%
April - July 2012	1,6,13	Ramik® 22.4	59	38	8	4	34	100%
April - July 2012	3,8,7	Rozol® 11.2	54	42	7	4	37	100%
April - July 2012	4,10,12	Rozol® 22.4	46	48	10	6	27	100%

Table 5. Unmarked mouse captures: comparison of edge (24) and interior (25) traps, and trapping night

Unmarked mice: edge vs. interior (24 vs. 25 traps)

			actual		expected	
date	plot #'s	bait	edge	interior	edge	interior
April - July 2012	2,5,9	Ramik® 14.4	2	2	2	2
April - July 2012	1,6,13	Ramik® 22.4	2	2	2	2
April - July 2012	3,8,7	Rozol® 11.2	4	1	2.5	2.5
April - July 2012	4,10,12	Rozol® 22.4	0	4	2	2
Combined	all		8	9	8.5	8.5

Combined	all		night 1	night 2	night 3	night 4
unmarked mice			6	3	2	4
proportion of catch			3.7	3.1	1.8	3.5

References:

Adler G.H, and R. Levins. 1994. The island syndrome in rodent populations. Quarterly Rev. Biol. 69:473–490.

Atkinson, I. A. E., 1985. The spread of commensal species of Rattus to oceanic islands and their effects on island avifauna. Pages 35-81 *in:* P.J. Moors (Ed.), Conservation of island birds. International. Council for Bird Preservation. Tech. Publ. 3. Cambridge, England.

Beauvais, G.P. and Steven W. Buskirk. 1999. Modifying Estimates of Sampling Effort to Account for Sprung Traps. Wildlife Society Bulletin. 27(1):39-43.

Brown, D. 1993. Eradication of mice from Allports and Motutapu Islands. Ecol. Manage. 1:19-30.

Buckle, A.P. and M.G.P. Fenn. 1992. Rodent control in the conservation of endangered species. Pages 36-51 *in:* J.E. Borrecco and R.E. Marsh (Eds.), Proceedings, 15th Vertebrate Pest Conf., Univ. Calif, Davis, CA.

Burbridge, A. and K. Morris. 2002. Introduced mammal eradications for nature conservation on Western Australian islands: a review. Pages 64-70 *in:* C. Veitch and M. Clout (Eds.), Turning the tide: the eradication of invasive species. SSC Invasive Species Specialist Group, IUCN, Gland, Switzerland.

Chelkowska, H. and L. Ryszkowski 1967. Causes of higher abundance estimates of small rodents at the edges of sampling areas in forest ecosystems. Ekol. Pols.,A 15:737-46.

Chitty, D. 1937. A ringing technique for small mammals. Journal of Animal Ecology. 6:36-53.

Courchamp, F., J.L. Chapuis and M. Pascal. 2003. Mammal invaders on islands: Impact, control and control impact. Biological Review 78:347-383.

Cuthbert, R.J. and G.M. Hilton. 2004. Introduced house mice Mus musculus, a significant predator of endangered and endemic birds at Tough Island, South Atlantic Ocean? Biological Conservation 117:483-489.

Dunlevy, P.A., E.W. Campbell III, and G.D. Lindsey. 2000. Broadcast application of a placebo rodenticide bait in a native Hawaiian forest. Inter. Biodeterioration and Biodegradation, 45:199-208.

Eason, C.T., G.R. Wright, L. Milne and G.A. Morriss. 2001. Laboratory and field studies of brodifacoum residues in relation to risk of exposure to wildlife and people. Science for Conservation Series 177b. Department of Conservation, Wellington, N.Z.

Eisemann, J.D. and C.E. Swift. 2006. Ecological and human health hazards from broadcast application of 0.005% diphacinone rodenticide baits in native Hawaiian ecosystems. Pages 413-433 *in:* R.M. Timm and J.M. O'Brien (Eds.), Proceedings, 22nd Vertebrate Pest Conf., Univ. Calif, Davis, CA.

Fitzgerald, B.M., B.J. Karl and H. Moller. 1981. Spatial organization and ecology of a sparse population of house mice (*Mus musculus*) in a New Zealand forest. J. Animal Ecology 50:489-518.

Gilles, C., A. Styche, P. Bradfield, K. Chalmers, M. Leach, E. Murphy, T. Ward-Smith and R. Warne. 2006. Diphacinone bait for ground control of rats on mainland conservation land. Science for Conservation Report 270. Department of Conservation, Wellington, N.Z., 20pp.

Gurnell, J. and J.H.W. Gipps. 1989. Inter-trap movement and estimating rodent densities. Journal of Zoology. 217(2):241-254.

Hadfield, M.G., S.E. Miller, and A.H. Carwile. 1993. The decimation of endemic Hawaiian tree snails by alien predators. American Zoologist 33:610-622.

Hess, S.C., C.E. Swift, E.W. Campbell III, R.T. Sugihara and G.D. Lindsey. 2009. Controlling small mammals. Pages 425--447 *in:* T.K. Pratt, C.T. Atkinson, P.C. Banko, J.D. Jacobi and B.L. Woodworth (Eds.), Conservation Biology of Hawaiian Forest Birds: Implications for Island Avifauna. Yale Univ. Press, New Haven, CN.

Johnston, J.J., W.C. Pitt, R.T. Sugihara, J.D. Eisemann, T.M. Primus, M.J. Holmes, J. Crocker and A. Hart. 2005. Probabilistic risk assessment for snails, slugs, and endangered honeycreepers in diphacinone rodenticide baited areas on Hawaii, USA. Environmental Toxicology and Chemistry 24(6):1557-1567.

Jones, A.G., S.L. Chown and K.J. Gaston. 2003. Introduced house mice as a conservation concern on Gough Island. Biodiversity and Conservation 12:2107-2119.

KIRC. 1998. Kahoolawe environmental restoration plan. Social Science Research Institute, Univ. Hawaii, Manoa. Report to Kahoolawe Island Reserve Commission (KIRC). 115pp.

Koehler, A.E., M.E. Tobin, M.J. Goodall, and R.T. Sugihara. 1995. Weatherability and acceptance of selected commercial zinc phosphide rodent baits. International Biodeterioration & Biodegradation. 36:35-50.

Lindsey, G.D., S.C. Hess, E.W. Campbell III and R.T. Sugihara. 2009. Small mammals as predators and competitors. Pages 274--292 *in:* T.K. Pratt, C.T. Atkinson, P.C. Banko, J.D. Jacobi and B.L. Woodworth (Eds.), Conservation Biology of Hawaiian Forest Birds: Implications for Island Avifauna. Yale Univ. Press, New Haven, CN.

MacKay, J.W.B., J.C. Russell and E.C. Murphy. 2007. Eradicating mice from islands: successes, failures and the way forward. Pages 294-304 *in:* G.W. Witmer, W.C. Pitt and K.A. Fagerstone (Eds.) Proceedings of an International Symposium: Managing Vertebrate Invasive Species. National Wildlife Research Center, Ft. Collins, CO.

Marsh, R.E. 1986. Role of anticoagulant rodenticides in agriculture. Presented at the Pacific NW Forestry and Orchard Animal Damage Control Conf., Washington State Univ., Oct. 27-30, Wenatchee, Washington. 10pp.

Marten, G.G. 1972. Censusing mouse populations by means of tracking. Ecology 53(5):859-867.

McCann, G.R. and G.H. Matschke. 2000. Standard house mouse anticoagulant dry bait laboratory test. Unpubl. Report. QA-506, National Wildlife Research Center, Ft. Collins, CO. 2pp.

McCann, G.R. 2000. Chlorophacinone and diphacinone: Standard *Mus musculus* and *Peromyscus maniculatus* anticoagulant laboratory tests. Pages 263-267 *in:* T.P. Salmon and A.C. Cragg (Eds.), Proceedings, 19th Vertebrate Pest Conf., Univ. Calif, Davis, CA.

Nelson, J.T., B.L. Woodworth, S.C. Fancy, G.D. Lindsey and E.J. Tweed. 2002. Effectiveness of rodent control and monitoring techniques for a montane rainforest. Wildl. Soc. Bull. 30(1):82-92.

Nelson, J.T. and F.W. Clarke. 1973. Correction for sprung traps in catch/effort calculations of trapping results. Journal of Mammalogy. 54(1):295-298.

Pitt, W.C., J. Eisemann, K. Swift, R. Sugihara, B. Dengler-Germain, and L. Driscoll. 2006. Diphacinone residues in free-ranging wild pigs following aerial broadcast of rodenticide bait in Hawaiian forests. Unpublished Report QA-1077. National Wildlife Research Center, Hilo, HI. 21p.

Pitt, W. C., L. C. Driscoll and R. T. Sugihara. 2010. Efficacy of rodenticide baits for the control of 3 invasive rodent species in Hawaii. Archives of Environmental Contamination and Toxicology 60(3):533-542.

Pitt, W. C., D. K. Foster, and R. T. Sugihara. 2012. Evaluation of candidate wildlife biomarkers to assess bait acceptance by mice (Mus musculus): comparison of biomarker detection and retention, and bait palatability. QA-1736 Final Report. USDA, APHIS, WS, NWRC. Hilo, HI. 32pp.

Rowe, F.P. and R. Redfern. 1968. Laboratory studies on the toxicity of anticoagulant rodenticides to wild house mice (*Mus musculus* L.). Proceedings, Association of Applied Biology 61(2):322-326.

Scott, J.M., S. Mountainspring, F.L. Ramsey, and C.B. Kepler. 1986. Forest bird communities of the Hawaiian Islands: Their dynamics, ecology, and conservation. Studies in Avian Biology 9.

Smith, M.H. 1971. Food as a limiting factor in the population ecology of *Peromyscus polionotus* (Wagner). Annales Zoologici Fennici. 8:109-112.

Spurr E.B., G.D. Lindsey, C. Forbes-Perry and D. Foote. 2002. Effectiveness of hand broadcast application of baits containing 0.005% diphacinone in reducing rat populations in Hawaiian forests. Pacific Island Ecosystems Research Center US Geological Survey, Unpubl. Report, QA-01.

Spurr, E.B., D. Foote, C. Forbes-Perry and G.D. Lindsey. 2003. Efficacy of aerial broadcast application of baits containing 0.005% diphacinone in reducing rat populations in Hawaiian forests. Pacific Island Ecosystems Research Center US Geological Survey, Unpubl. Report, QA-02.

Stenseth N. C. and Hansson, L 1979. Correcting for the edge effect in density estimation: explorations around a new method. Qikos 32:337-348.

Sugihara, R. T. 1997. Abundance and diet of rats in two native Hawaiian forests. Pacific Science 51(2):189-198.

Swift, C.E. 1998. Laboratory Bioassays with wild-caught black (Rattus rattus) and Polynesian (R. exulans) rats to determine minimum amounts of Ramik Green® (0.005% diphacinone) and exposure times for field broadcast applications in Hawaii. M.S. thesis. Univ. Hawaii, Honolulu. 88 pp.

Taylor, R.H. and B.W. Thomas. 1993. Rats eradicated from rugged Breaksea Island (170ha), Fiordland, New Zealand. Biological Conservation 65:191-198.

Tobin, M. E. 1994. Mitigation of rat depredation in native Hawaiian habitats. Transactions, Western Section Wildlife Society. 30:15-20.

Tobin, M. E., Koehler, A. E. and Sugihara, R. T., 1996. Comparison of bait markers for black rats. J. Wildl. Manage. 60: 202-207.

Wanless, R.M., A. Angel, R.J. Cuthbert, G.M. Hilton and P.G. Ryan. 2007. Can predation by invasive mice drive seabird extinctions? Biology Letters 3:241-244.

Witmer, G., P. Burke, S. Jojola and P. Dunlevy. 2006. The biology of introduced Norway rats on Kiska Island, Alaska, and an evaluation of an eradication approach. Northwest Science 80:191-198.

Williams, J. 1990. The coastal woodland of Hawaii Volcanoes National Park: vegetation recovery in a stressed ecosystem. Technical Report 72. Cooperative National Park Resources Studies Unit. Univ. of Hawaii, Manoa, Honolulu, Hawaii.

Witmer, G. W. 2001. Captive Canada geese acceptability and toxicity trials with two formulations of 0.005% diphacinone rodenticide baits. Unpublished report, QA-770, USDA National Wildlife Research Center. 66 pages.

Witmer, G. 2007. Efficacy of commercially available rodenticde baits for the control of wild house mice. Final report, QA-1304. National Wildlife Research Center, Ft. Collins, CO. 16pp.

Young, L., C. Miller, E. VanderWerf, T. Takahama, J. Hatakenaka, D. Anderson, H. Leiong, P. Dunlevy, C. Swenson and B. Liesemeyer. 2009. Relative abundance, reproductive cycle and homer range of rodents in Kaena Point Natural Area Reserve. Poster presentation at 2009 Hawaii Conservation Conference, July 28-30, 2009, Honolulu, HI.

Xuhua, Xia and Rudy Boonstra. 1992. Measuring Temporal Variability of Population Density: A Critique. The American Naturalist 140(5):883-892.