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RESEARCH ARTICLE

Assessing spatial variation and overall density of aerially broadcast toxic bait during a rat eradication on Palmyra Atoll

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Abstract Baits containing brodifacoum rodenticide were aerially applied to eradicate invasive black rats from Palmyra Atoll, an important biodiversity center. Bait application must be sufficient to be effective, while minimizing environmental hazards by not exceeding designated label rates, prompting our bait density assessments for two aerial drops. With few physical or human resources on this remote, uninhabited atoll, assessments were particularly challenging, requiring observations within 30 min of aerial application to avoid bait loss to rats, crabs, or elements. We estimated bait density using quadrat sampling within 13 terrestrial sampling areas. We also sampled 10 tidal flat areas to assess inadvertent bait scatter into marine aquatic environments. Of particular value for challenging sampling circumstances, our quadrats had to be lightweight and durable, which we addressed by using widely available PVC hoops ("Hula Hoops"), the size of which was ideal for sampling purposes. At 77.5 and 78.7 kg/ha, overall bait densities were very near to the target densities of 80 and 75 kg/ha, respectively. However, considerable variability in bait densities existed among sampled areas, 8.6–178.2 and 31.4–129.5 kg/ha for the respective drops. Environmental, human, and equipment factors likely accounted for this variability. Tidal flat sampling revealed variable bait scatter into aquatic environments, from 0–46.3 kg/ha across the two drops. No differences were found in average bait densities among 1-, 4-, and 7-m distances from high tide lines. Our methods might broadly assist bait density (and other) surveys under challenging circumstances.

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Introduction

The health of ecosystems can be severely impacted by invasive species, with the negative impacts inflicted by exotic species on native species and ecosystems possibly exceeded only by human-caused habitat destruction (Parker et al. 1999; Wilcove et al. 1998). In particular, island ecosystems around the world have suffered severely from invasions by rats (Atkinson 1985; Campbell and Atkinson 2002; Howald et al. 2007; Jones et al. 2008; Towns et al. 2009; Veitch and Clout 2002). The destructive impacts of invasive species motivate management actions, but these actions must be assured to also not present unreasonable hazards to the environment. As a specific example, the application of pesticides (rodenticide baits in this example) targeting an invasive species must be carefully evaluated to not only ensure efficacy (100 % in the case of an eradication) but to also ensure no unreasonable adverse effects on the environment (Federal Insecticide Fungicide and Rodenticide ACT, Section 3(a)). Such was the case on Palmyra Atoll where introduced black rats (*Rattus rattus*) had been impacting the terrestrial ecosystems since World War II and where a recent eradication effort was reliant on aerial applications of rodenticide baits.

Palmyra Atoll is the northernmost island complex of the Line Island Archipelago, approximately 5° north of the equator and 1,600 km south of Hawaii. This atoll, comanaged by the U.S. Fish and Wildlife Service and The Nature Conservancy, is an important center of biodiversity and species abundance and notably is the only seabird nesting habitat within ca 725,000 km^2 of ocean and an important marine feeding ground for seabirds (U.S. Fish and Wildlife Service 2011). Black rats have degraded nearly all facets of Palmyra Atoll's ecosystems, from breeding seabirds to the native *Pisonia* forest ecosystem (Flint 1999; Wegmann 2009). They appear responsible as a limiting factor in seabird nesting success (Flint 1999; U.S. Fish and Wildlife Service 2011). They also attack hermit crabs (*Coenobita* spp.), land crabs (*Cardisoma* spp.), and coconut crabs (*Birgus latro*); kill native plant seeds and disperse them to where they will not germinate (Wegmann 2009); and may pose a threat to sea turtle hatchlings as has occurred elsewhere (Caut et al. 2008).

Rat removal on Palmyra Atoll was expected to provide biodiversity benefits for seabirds, plants, terrestrial invertebrates, and many other components of its terrestrial ecosystems, and a significant component of the biosecurity plan for Palmyra Atoll included eradicating black rats (Hathaway and Fisher 2010). Around the world, rat eradication typically has resulted in increased productivity for bird populations (Lavers et al. 2010) and, moreover, has been documented to aid in indigenous forest restoration (Allen et al. 1994).

A rat eradication effort was initiated in 2011 using an integrated management approach centered on two aerial applications (and two application rates) of grain-based pellet bait containing 25 ppm of the second generation anticoagulant rodenticide brodifacoum (Brodifacoum 25W: Conservation, EPA Reg. No. 56228-36, Supplemental Label-Palmyra Atoll). These ca 1-cm diameter × 1.5-cm cylindrical, green-colored baits are manufactured specifically for conservation purposes. The aerial applications were also supported by localized specialized baiting, which included placement of bait stations around U.S. Fish and Wildlife Service/The Nature Conservancy (USFWS/TNC) structures (cabins, galley, etc.) and hand baiting in historic structures (i.e., WW II era military bunkers, pill boxes, etc.) and in tree canopies. It is noteworthy that the supplemental label for this bait created specifically for this operation allowed for 4.4 and 8.3 times the bait densities as the parent label, respectively, for the first and second broadcast bait applications.

It is crucial that baits are applied according to a plan that ensures sufficient bait to achieve desired efficacy, while minimizing environmental hazards by ensuring label application rates are not exceeded, an example of which occurred at Rat Island, Alaska (Salmon et al. 2010). Independent verification of aerial bait application rates can assure not only that bait label limits are not exceeded but also that baiting efforts meet bait density objectives. We conducted an independent assessment of the aerial bait application rates for the rat eradication operation on Palmyra Atoll. The remoteness of Palmyra Atoll, the relative lack of infrastructure, and the lack of readily available human and physical resources necessitated methods to monitor bait application to be as labor efficient as possible, while simultaneously delivering high-quality data and accurate analytical inferences. Here, we describe a bait density assessment conducted under difficult physical (intense activity in hot, humid conditions on a landscape where mobility is impeded by lush vegetation) and temporal (within half hour of bait drops) constraints, with sampling procedures facilitated by employing an inexpensive, lightweight, highly portable means to delineate sampling quadrats.

Study site

The approximately 2.5-km^2 land area of Palmyra Atoll is comprised of ca 50 islets ranging from about 0.1 to 97.9 ha in size and arrayed in a horseshoe configuration around a central lagoon system surrounded by coral reef (Collen et al. 2009). The islets have a maximum elevation of 2 m and are generally heavily vegetated with a dense canopy cover. The atoll is dominated by an overstory of coconut (*Cocos nucifera*) palms. The terrestrial ecosystem supports some of the best remaining protected *Pisonia grandis* forest as well as patches of *Scaevola taccada* (Naupaka) and *Lepturas sp.* Average rainfall is approximately 440 cm per year and daytime temperatures average 29 °C year round. The atoll is a home to large colonies of seabirds, including the second largest colony of red-footed boobies (*Sula sula*) in the world, as well as significant numbers of overwintering shore birds, including bristle-thighed curlews (*Numenius tahitiensis*) and Pacific golden plovers (*Pluvialis fulva*), both of which are designated as species of high conservation concern (Flint 1999; Engilis and Naughton 2004). The atoll was heavily modified by the U.S. Navy during World War II, and remnants of bunkers, gun embankments, and pill boxes are distributed throughout the islets. Two islets (Quail and Barren Islands) are off-limits to foot traffic due to the continued presence of unexploded military ordinance.

Methods

Bait application

The bait application operation was conducted by a private contractor (Island Conservation, Santa Cruz, CA). The two bait drops were conducted on 12–16 June and 21–22 June, 2011, which is during the time when the seabird and shorebird populations are at their lowest (U.S. Fish and Wildlife Service 2011), thereby minimizing risk of bird exposure to the rodenticide. All emergent land areas were treated by a helicopter during each of two aerial applications of bait over the entire atoll, with a target application rates of 80 and 75 kg/ha, respectively. Aerial baiting included broadcast distribution using three application methods (U.S. Fish and

Wildlife Service 2011): (1) full swath (40 m) for interior baiting, (2) a directional swath (20 m) with a deflector to broadcast bait to the onshore side of the helicopter while applying bait along shore lines (above the high tide line), and (3) a narrow swath (5 m) for baiting land areas between 10 and 25 m wide or for gaps in coverage.

In some locations, supplementary bait application (<5 % of total bait distributed) was performed by hand baiting and establishment of bait stations, both of which were performed per label requirements. Hand baiting was conducted around buildings operated by USFWS/TNC as well as in abandoned military structures such as bunkers, pill boxes, and gun embankments. Bait stations were established inside dwellings currently in use by USFWS/TNC staff and other crew. Baiting of coconut palms was conducted by placing two cotton sacks, each filled with 12.5 g bait and connected by 50-cm twine directly into the coconut palm crowns either by handheld catapults or dropped by an applicator suspended below a helicopter.

Our charge was specifically to assess the aerial broadcast bait application rates for the two aerial broadcast bait applications, with sampling occurring in real time within a half hour of bait being dropped.

Bait sampling areas

To ensure the representation of the components of the fragmented land area and the habitat types found within Palmyra Atoll, 13 terrestrial areas throughout the atoll were sampled for assessing aerial bait application rate (Fig. 1). Most areas had a large proportion covered by vegetative canopy (the only sampling area with no canopy cover was North Runway). Ten tidal flat locations around the atoll (Fig. 2) were sampled during aerial baiting operations to determine if and how much bait intended for terrestrial targets landed in marine aquatic environments. The tidal flats are long and shallow, thereby providing opportunity to sample while the tide is out.

Sampling quadrats

Quadrat sampling is well known to provide unbiased estimates (e.g., Engeman et al. 1994; Krebs 1999) and was applied within each of the terrestrial and tidal flat sampling areas to collect the data from which to estimate bait density. Circular quadrats have the lowest perimeter to edge ratio (e.g., Krebs 1999) and were used to reduce decision making in the field on whether baits directly on the quadrat perimeter were to be considered as in the quadrat.

To ensure that observations were made prior to bait removal by rats, crabs, or the elements, sampling crews were followed as closely behind the helicopter as safely possible, within 30 min of aerial baiting. Thus, it was essential that sampling frames be constructed of durable and easily carried and handled lightweight material. We used circular PVC hoops (i.e., “Hula Hoops”) to delineate sampling quadrats. We considered the size of the quadrat to be defined as the exterior diameter of the hoop. The PVC hoops used for terrestrial sampling quadrats were 1 cm thick and had an exterior diameter of 89 cm. Given the projected bait distribution rates and given that sampling logistics made locating and observing the sample points about equal in time and effort, the size of our quadrats was about optimal for the circumstances (see, for example, Wiegert 1962). The same type of sampling hoop used in the terrestrial sampling was used at 6 of 10 tidal flat sampling areas. At the other four tidal sampling areas, a slightly larger hoop was used (due to limited quantities of hoops), with a hoop thickness of 1.5 cm and an exterior diameter of 90.5 cm. Bait pellets were considered inside the PVC hoop if any portion of the pellet was inside the area bordered by the inner perimeter of the PVC hoop. This definition for including the baits partially bordering the inner perimeter served to effectively resolve decision making in the field about which baits directly under the frame to include in the sample. For unbiased quadrat sampling, half the baits in contact with the quadrat boundary should be included and half excluded. Our method testing showed that about half of the baits contacting the exterior perimeter of the hoop also contacted the interior perimeter and therefore were included as inside the quadrat.

Terrestrial sampling methods

Thirteen terrestrial sampling areas were established throughout the atoll prior to bait application. Within approximately 30 min following bait application at the sampling area, a crew member entered each sampling area, which had been spatially stratified to insure sampling coverage throughout. Sampling points were randomly selected by entering each stratum at a random start point and walking a random distance, at which point, the crew member faced north, dropped a hoop from shoulder height and allowed it to settle on the soil surface for observation. Subsequent to data collection, the crew member set off in a random direction for a random distance >5 m and repeated the hoop drop until all strata within an area were sampled. Fifteen such hoop drops total were conducted in each terrestrial sample area. All bait pellets (or fractions of a pellet) were removed from the area within the PVC hoop as defined above and placed in a bag labeled with a unique identification number, date, place of collection, and the collector's identity.

Tidal flat sampling methods

Sampling for bait missing the terrestrial target and landing in each of the 10 tidal flats was conducted while the tide was

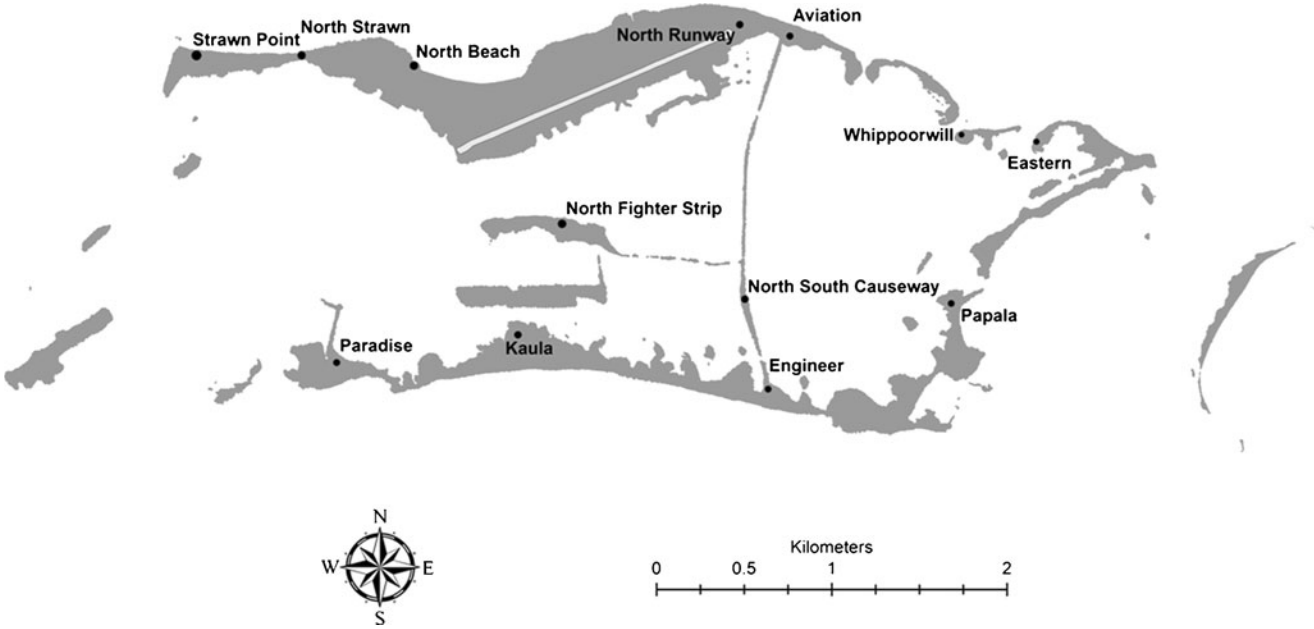

Fig. 1 A map of the 13 sampling locations used to measure terrestrial bait application rates during each of the two aerial broadcasts during the Palmyra Atoll rat eradication

out using a grid of 15 PVC hoops, structured with three rows of five quadrats (hoops) centered at distances from the high tide line of 1, 4, and 7 m, with 5 m between row members (Fig. 3). This technique allowed for fine-scale examination of bait scatter from the terrestrial target. Each plot was identified and flagged with biodegradable flagging, and hoops were staged above the high tide line >24 h prior to bait application. Crew members were stationed at plot locations approximately 1 h prior to commencement of bait application, and hoops were then arranged into the grids. This allowed sampling crews to optimize activities of following as close as possible during a receded tide as the helicopter baited the adjacent terrestrial habitat.

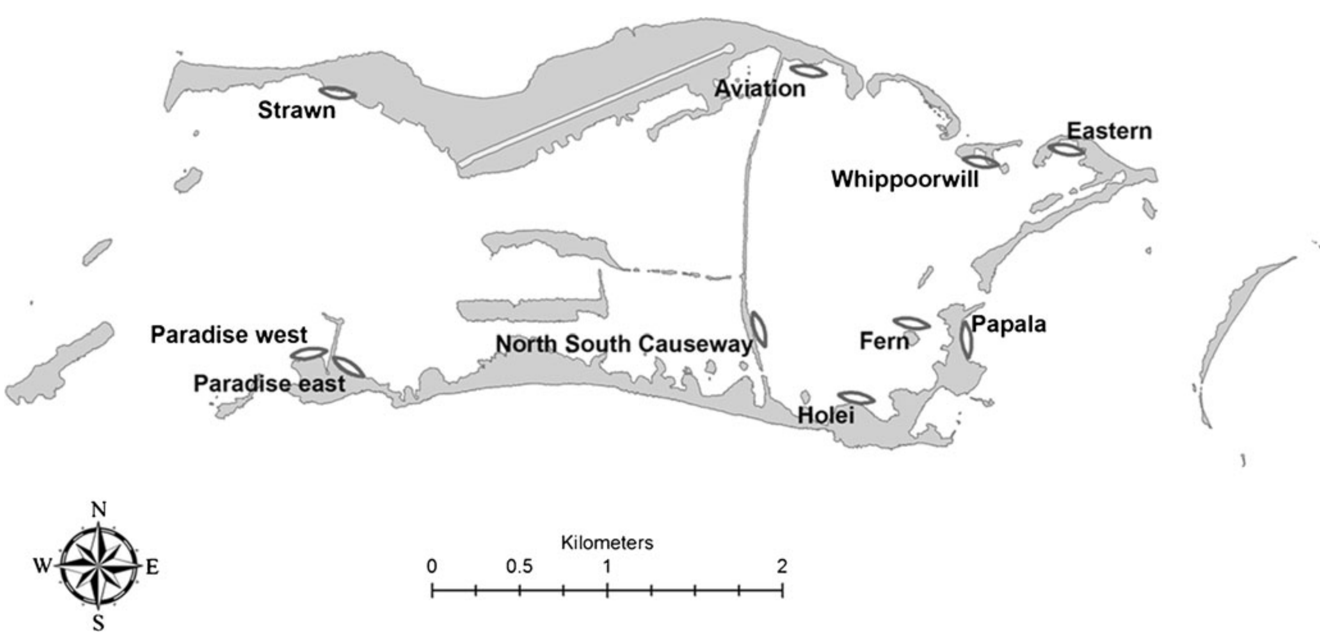

Fig. 2 A map of the 10 sampling locations used to measure bait scatter into the near-shore marine environment during each of the two aerial broadcasts during the Palmyra Atoll rat eradication

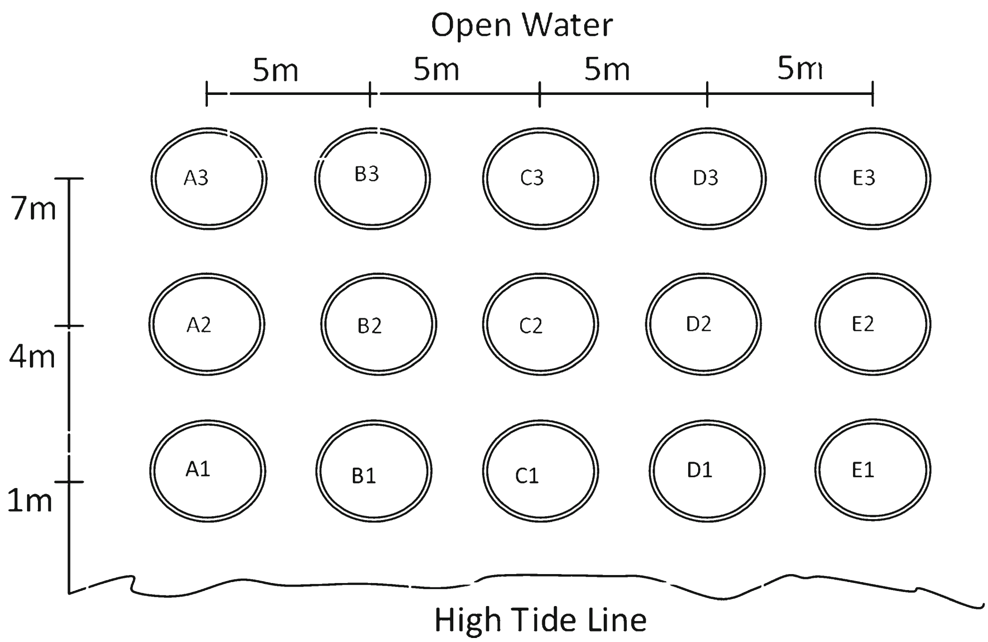

Fig. 3 Grid schematic (not to scale) for the layout of 15 quadrats in three rows of five at distances of 1, 4 , and 7 m from the high tide line, with this layout used at each of 10 sampling locations for measuring bait scatter into the near-shore environment

Bait handling

During sampling, all personnel complied with label requirements for the use of personal protective equipment. Bait particles were weighed in a small laboratory on Palmyra Atoll using a portable scale with an accuracy to 0.01 g. In the event that bait particles got wet due to frequent rains, they were frozen at −80 C and transported at the conclusion of the study to the USDA/APHIS/WS/NWRC field station in Hilo, Hawaii. Wet bait particles were then dried in a commercial drying oven at 38–42 °C until dry (24–48 h) and weighed after cooling.

Data analyses

Quadrat sample density estimates were calculated using the standard formula (e.g., Engeman et al. 1994): $\Sigma x_i/(aN)$ where x_i is the observation from the ith quadrat, a is the quadrat area (constant area for each quadrat within each sample area, otherwise it would be subscripted), and N= 15, the sample size taken at each area. Terrestrial bait densities were expected to vary spatially among the terrestrial sampling areas due to differing levels of canopy cover, wind speed and direction, and positioning of baiting swaths in an irregular landform. The overall aerial bait density for the atoll was calculated by averaging the bait densities from the 13 sampling areas. The variance for this overall estimate was calculated to account for area-to-area variability as well as heterogeneous within-area variances. For assessing bait scatter into the marine aquatic environment, we compared bait densities among the three distances from shore using a randomized blocks ANOVA with aquatic sampling areas serving as the blocking component.

Results

Terrestrial bait density sampling

Estimated bait densities from each of the terrestrial sampling areas within each bait application are summarized in Table 1. The overall bait densities for the first and second drops were 77.5 kg/ha (standard error (SE)=23.4) and 78.7 kg/ha (SE= 17.1) and were only −2.5 and +3.7 kg/ha from the target densities of 80 and 75 kg/ha, respectively. However, a high degree of variability for bait densities was exhibited among

Table 1 Terrestrial bait density results from 13 terrestrial sampling areas on Palmyra Atoll (N=15 samples/area)

Sampling area	First application 80 kg/ha target, bait density (SE)	Second application 75 kg/ha target, bait density (SE)
Aviation	N/A	111.4 (14.3)
Causeway	45.0 (12.1)	63.7 (15.7)
Eastern	77.3 (14.5)	82.0 (19.3)
Engineer	50.2 (10.2)	53.0 (14.9)
Fighter Strip	19.2 (6.3)	31.4 (9.7)
Kaula	178.2 (20.8)	129.5 (20.0)
North Beach	8.6 (3.8)	77.5 (15.6)
North Runway	69.0 (9.7)	126.7 (18.1)
North Strawn	43.4 (10.3)	92.1 (12.1)
Papala	72.1 (16.6)	39.0 (11.3)
Paradise	146.9 (38.2)	103.3 (17.2)
Strawn Point	50.2 (9.6)	55.6 (12.1)
Whippoorwill	169.7 (25.7)	57.5 (13.0)
Mean over all areas	77.5 (23.4)	78.7 (17.1)

N/A not available

the sampling areas across the atoll for both drops (Table 1). During the first bait drop, an equipment failure caused early termination of baiting activities over the Aviation sampling area. Baiting was completed the following day, making bait samples inconsistent with other areas, because it was impossible to determine how much of the already distributed bait had been removed by rats and crabs between the 2 days. Among the other 12 areas, bait density ranged from 8.6 kg/ha at the North Beach sampling area to 178.2 kg/ha at the Kaula sampling area. For the second bait drop, bait density ranged from 31.4 kg/ha at the Fighter Strip sampling area (which had the second lowest density in first drop) to 129.5 kg/ha at the Kaula sampling area, which also had the highest bait density from the first drop.

Tidal flat bait density sampling

As with the terrestrial results, there also was a great deal of variability in bait density among the aquatic sampling areas (Table 2). For the first drop, bait density averaged over the three sampling distances from the high tide line ranged from a barely detectable 0.4 kg/ha at Paradise East to a maximum of 44.7 kg/ha at the Aviation aquatic area. Differences in bait density were not detected among the three distances from the high tide line ($F_{2,18}$=2.08, p=0.15). The shortest distance (1 m) from the high tide line had the highest bait density for 6 of the 10 areas, the middle distance (4 m) had the highest bait density for 3 of the areas, and the furthest distance (7 m) had the highest bait density for 1 area (Table 3). Similar results characterized the second drop, although one area (Paradise West) could not be sampled because it was flooded. Bait density averaged over the three distances from the high tide line ranged from 0.0 kg/ha at Papala aquatic to 46.3 kg/ha at Aviation aquatic, which again had the highest scatter of bait into a tidal flat. Again, no differences in bait density were observed among the three sampling distances from the high tide line ($F_{2,16}$=1.13, p=0.35). For this drop, the 1-m distance from the high tide line had the highest bait density for four of the nine areas, the 4-m distance had the highest bait density for two of the areas, the 1- and 4-m distances had essentially the same bait density for Strawn aquatic, and none of the three distances had any bait observed at Papala aquatic (Table 3).

Table 2 Marine aquatic bait density sampling results from 10 tidal flat areas to assess bait scatter from terrestrial targets on Palmyra Atoll (N=15 samples/area arranged in three rows of five samples spaced at 1, 4, and 7 m from high tide line)

Sampling area (all distances from high tide line combined)	First application (terrestrial target = 80 kg/ha), bait density (SE)	Second application (terrestrial target = 75 kg/ha), bait density (SE)
Aviation aquatic	44.7 (11.5)	46.3 (6.0)
Causeway aquatic	3.2 (2.3)	2.3 (2.3)
Eastern aquatic	8.5 (3.8)	9.5 (6.2)
Fern aquatic	24.9 (8.1)	22.4 (9.1)
Holei aquatic	24.4 (8.1)	30.1 (6.6)
Papala aquatic	8.5 (4.6)	0.0 (0.0)
Paradise East aquatic	0.4 (0.4)	9.0 (4.0)
Paradise West aquatic	3.8 (2.9)	flooded
Strawn aquatic	33.4 (8.2)[a]	18.9 (7.7)
Whippoorwill aquatic	10.0 (4.8)	24.3 (10.2)

[a] The directional deflector broke while the land adjacent to the Strawn aquatic plot was being baited

Table 3 Marine aquatic bait density sampling results from 10 tidal flat areas to assess bait scatter from terrestrial targets at three distances from the high tide line on Palmyra Atoll

Distance from high tide line (m)	First application (terrestrial target = 80 kg/ha), bait density (SE)	Second application (terrestrial target = 75 kg/ha), bait density (SE)
1	22.0 (4.5)	22.7 (4.5)
4	15.5 (4.5)	17.3 (4.1)
7	11.0 (2.9)	14.3 (4.0)

Discussion

The overall average accuracies of the two bait drops well approximated the target densities for the atoll as a whole. However, there was considerable variability among bait densities across the sampling areas, for both the terrestrial and aquatic ecosystems. Under some circumstances, localized areas of under- or over-baiting could present problems. Under-baiting or gaps in bait application might essentially create refuge areas where not all rats would be exposed to bait, thereby undermining the eradication effort. As already indicated, plans were built into the eradication protocol to address areas that might be shielded from the aerial drops.

Over-baiting could present short-term risks to native fauna. As described earlier, baiting was conducted when the seabird and shorebird populations are at their lowest so minimal numbers would be exposed to bait. Thus, the primary native land animals exposed to localized areas of higher bait density would be crabs and insects, but the risks would likely be short lived for these bait matrices in the hot, humid, and rainy environment of Palmyra Atoll (Dunlevy et al. 2000).

A number of factors likely contributed to the wide variability observed in bait densities among areas. Very precise baiting swaths were devised using GIS mapping and GPS implementation, with real-time adjustments made during operations. The difficulty in developing and applying baiting swaths to Palmyra Atoll while avoiding spillage into the aquatic environment is readily perceived by viewing the irregular land forms in Fig. 1. Swath overlap, windblown baits between swaths, the effects of canopy cover, and the interaction among these factors might account for terrestrial areas receiving varying bait densities. The precise reason for the higher bait density at the Kaula terrestrial area remains unknown but may be related to overlapping bait swaths. We note that the EPA label obtained specifically for this project accounted for areas of swath overlap and other unintended forms of higher-than-target bait densities by allowing the limit for total bait distribution to be ≤47,000 kg for the entire operation (Brodifacoum 25W: Conservation, EPA Reg. No. 56228-36, Supplemental Label-Palmyra Atoll).

For bait scatter into marine aquatic areas, such as Aviation aquatic which had the highest aquatic bait density during both bait drops, a variety of possible confounding factors all could have contributed to the considerable variability among sampled areas. Directional baiting is used to limit the amount of bait that enters the aquatic environment as the pilot flies parallel to the shoreline. Our data indicate that sampling quadrats furthest from shore (7 m) still contained 14 to 19 % of the target application rate on average, and up to 62 % of the target terrestrial bait density was delivered to the sampled aquatic environments within 7 m of the high tide line. The factors contributing to these results might include baits drifting in the wind, a lack of efficacy of the directional bait deflector on the helicopter, and pilot difficulties in accurately determining the location of the shoreline due to overhanging coconut palms. During the first bait application, the directional deflector broke while the land adjacent to the Strawn aquatic plot was being baited, thereby contributing to the second highest bait density among the aquatic areas for that application (33 kg/ha, Table 2). Moreover, dense canopy cover that includes palm trees overhanging the shoreline could have deflected some bait pellets beyond the high tide line at some aquatic sampling areas.

Results from the North Runway area were of interest because this is the only sampling area without a canopy cover, meaning all bait fell to the ground without hanging up in a canopy. The bait application rates for this area were 69.0 and 126.7 kg/ha, respectively, for the two drops (Table 1). Because considerable variability was exhibited for this area relative to obtaining the target bait rates (−11 and +51.7 kg/ha, respectively, for the two drops), it appears impossible to use this canopy-free sampling area as a reliable baseline for assessing the amount of bait caught in the canopies of sampling areas with substantial overstories. More to this point, eight of the other terrestrial sampling areas (Table 1: Causeway, Fighter Strip, Kaula, North Beach, North Strawn, Papala, and Whipoorwill) showed a high degree of variability in the relative amounts of bait received between the two drops, further indicating the difficulty in separating out effects of canopy hang-up from variability in bait distribution. Unfortunately, we did not have the resources to measure bait hang-up in canopies, but we recommend such endeavors be included in future application rate-monitoring programs. For the same reasons, no valid means existed to analyze the effect of canopy type on observed bait density on the ground.

As a final note, we comment briefly on the sampling techniques we employed. The mechanisms we used to apply quadrat sampling optimized the labor and provided a context for obtaining quality observations in difficult field situations with severe temporal sampling constraints. To ensure that samples were collected within one-half hour of bait being dropped, our sampling crews had to place quadrats and collect data while the helicopter was in the air distributing baits. Thus, materials had to be lightweight and strong to enable greatest mobility of the personnel and durability of the apparatus. For situations where multiple bait drops are to be made, our sampling procedures with the lightweight, sturdy frames provide the potential for rapidly collecting data from a drop which in turn can provide information on bait density and distribution for refining procedures for subsequent bait drops. As such, we made our sampling data available to the baiting operators, in case they could use the information for follow-up spot baiting in the areas having unacceptably (as determined by the operators) low bait densities. Our quadrat materials can be purchased at stores around the world, making them almost universally available. Of course, the size and shape of quadrats will vary among applications (e.g., Krebs 1999), but we envision that our materials and methods could be a valuable, inexpensive, and readily available resource for many applications the world over. The use of our procedures, including the PVC hoops, might well facilitate or make possible other bait density surveys where baits are aerially dispensed under challenging circumstances and/or where samples must be acquired rapidly after bait application.

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