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ABSTRACT: Rodent eradications have contributed to the recovery of many threatened species, but challenges often exist for campaigns that occur on tropical islands when compared to more temperate regions. A post-operational review of a rat eradication operation on Wake Atoll indicated that certain areas, such as those with high alternative food abundance, may have contributed to the failure to remove all Polynesian rats. We conducted a nontoxic bait uptake trial to evaluate whether the maximum prescribed bait application rate for Brodifacoum-25W rodenticide pellets was sufficient to expose all rats to a lethal dose at three sites on Wake Atoll, including around a solid waste aggregation area (SWAA), which was previously identified as "high risk." We monitored bait persistence and condition throughout the treatment period as well as rat movement via radio tracking. Bait uptake by rats was also assessed by trapping and examination of rat orifices and gastrointestinal contents for pyranine biomarker incorporated into the bait pellets. The rate of bait disappearance differed by site, with bait disappearing the fastest in vicinity of the SWAA. Rat movement also varied by site, with rats observed traveling greater distances around the SWAA, sometimes exceeding 300 m. The SWAA was the only site at which we observed rats negative for biomarker exposure. We suggest that these negative observations resulted from lack of bait availability or movement of rats into the core trapping area from outside the treatment area. However, we cannot rule out preferential selection of alternative food sources over bait pellets and suggest that this possibility should receive further attention. Based on our results, we conclude that, of the three sites, the maximum bait application rate prescribed on the product label was not high enough to provide every rat an opportunity to encounter bait at and around the SWAA. Given the rapid disappearance of bait and the regular immigration of rats from distant habitat, we recommend that an even greater application rate be prescribed and that the heavier treatment be extended over a much larger area surrounding the SWAA.

KEY WORDS: Asian house rat, bait availability, bait persistence, brodifacoum, conservation, invasive species, island restoration, Polynesian rat, radiotelemetry, *Rattus exulans*, *Rattus tanezumi*, rodent control, rodenticide, tropical, Wake Atoll

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INTRODUCTION

Invasive rodents are a threat to island ecosystems and biodiversity world-wide (Mulder et al. 2009, Towns 2009). Rodent eradication techniques have been developed in response to this threat and can help the recovery of many threatened species (Howald et al. 2007, St. Clair et al. 2011). The majority of successful eradication attempts have incorporated the use of anticoagulant rodenticides (Howald et al. 2007). Eradication attempts on tropical islands have had higher failure rates than in temperate regions (Russell and Holmes 2015).

In May 2012, an attempted eradication of the invasive *Rattus tanezumi* (Asian house rat) and *R. exulans* (Polynesian rat) took place on Wake Atoll. Although it appears that *R. tanezumi* was successfully eradicated, *R. exulans* populations have since recovered and are once again abundant (Griffiths et al. 2014). The presence of rats on Wake Atoll negatively impacts the native flora and fauna (including breeding seabirds, native plants, and native invertebrates) as well as services provided by various U.S. government agencies on island. The 2017 Wake Island Airfield Integrated Natural Resources Management Plan calls for follow-up eradication efforts based on lessons learned from the 2012 operation and additional information obtained since those efforts (USAF 2017). Our bait uptake trial was one of the efforts required to establish confidence in prospects for a future successful eradication action by the USAF. Specifically, this study was designed to provide answers to the following questions:

1) Is the label-prescribed application rate for the selected bait type high enough to provide every rat within the project boundary with an opportunity to consume a lethal dose of bait?
2) Will all rats within the project boundary consume bait despite access to natural and commensal food sources available at the time of the study?
3) How fast do rodenticide bait pellets disappear when applied to differing habitat types on Wake Atoll?

METHODS

Study Site

We conducted the study from 27 October to 24 November 2017 on Wake Atoll, an unincorporated territory of the United States in the central Pacific Ocean managed by the U.S. Air Force. Three treatment areas were established in different locations on Wake Island, to represent the diversity of habitats found on Wake Atoll and to test bait uptake and persistence in high rat density areas. Treatment Area 1 represented a mixed shrub/grassland habitat,

Treatment Area 2 represented closed-canopy forest vegetation, and Treatment Area 3 was situated in a solid waste aggregation area (SWAA), representing very high rat density. Boundaries for Treatment Areas 1-3 were chosen to utilize natural shoreline barriers with each total areas of approximately 10 ha (site maps available in Niebuhr et al. 2018).

Bait Product

For this trial application, we used a nontoxic version of cereal-based Brodifacoum-25W Conservation ½-inch pellets (B-25W; Bell Laboratories, Inc., Madison, WI). B-25W (0.0025% brodifacoum) is a second-generation anticoagulant rodenticide and is the primary aerially-applied bait product used in U.S. island eradication efforts, with an established record of high efficacy. The nontoxic version we used in this study also contained the inert biomarker pyranine, which fluoresces under UV light. The product label for B-25W prescribes a maximum bait application rate of 18 kg/ha for the 1st application followed 5-7 days later by a 2nd application of 9 kg/ha.

B-25W was used in the 2012 eradication attempt on Wake Atoll. The post-operation review of eradication failure (Brown et al. 2013) cited complexity of the Wake baiting operation, possible gaps in bait availability in commensal and intertidal areas, and availability of alternative food sources as likely causes of the eradication failure. Previous work (Shiels et al. 2015) showed reasonable acceptance of B-25W compared to Diphacinone-50 (0.005% diphacinone, Hacco Inc., Madison, WI) and natural alternative food sources. Given the current lack of an available alternative bait matrix proven to be more palatable to *R. exulans* and the higher success rate of operations employing brodifacoum, we chose to proceed with this product as a reasonable surrogate for future rodenticide use on Wake; however, the determination of what rodenticide will be used for future eradication efforts has not yet been made.

Treatment

We adhered to the label-prescribed maximum bait application rates for this study. Bait was hand broadcast by four applicators walking adjacent transects (10 m apart) and evenly distributing (i.e., throwing) bait 5 m on either side, from start to finish. The timing of each of the second applications was based on daily observations of bait persistence within the label guidelines. All bait handling, including application and monitoring, was conducted with gloved hands to minimize potential biases associated with human scent.

Bait Monitoring

We monitored bait on the ground daily for persistence and condition at four bait monitoring locations within each treatment area. These bait monitoring plots were established on day 0 of the first application, within an hour after each broadcast.

Bait Persistence Plots

Our bait persistence plots consisted of four 3-m^2 plots used to measure the changes in available bait on the ground over time. Each day, we collected, weighed, and replaced all bait pellets and large pieces. We monitored plots in this manner until no bait was recorded following the second application. Prior to the first measurement following each application, we moved any piece of bait that was close to (but outside of) each persistence plot at least 1 m away to prevent later inclusion in measurements due to confusion.

Bait Exclusion Plots

We established four bait exclusion plots to observe bait condition over time and after exposure to weather events (e.g., rainfall). Each plot consisted of six bait pellets placed in wire mesh cages, within which pellets were exposed to the elements but protected from consumers. These exclusion plots were maintained throughout both applications without replacing the pellets. Two additional exclusion plots were established on day 0 of the second application to collect data on the newer, fresher bait pellets. Bait condition was scored daily on a scale from 1 to 7 (Table 1).

Bait Uptake Evaluation

Subsequent to the second bait application, we assessed bait uptake by rats in each treatment area. Rats were trapped within the core of each treatment area using a combination of Haguruma (Haguruma, Osaka, Japan) and Sherman traps (HB Sherman Traps, Inc., Tallahassee, FL) baited with coconut as well as barrel traps baited with peanut butter. We established rat sampling areas within a minimum buffer of 150 m from any unbaited habitat outside the treatment area, with shoreline forming part of the buffer in some areas. After the second bait application, we positioned closed traps throughout the sampling areas to allow time for the rats to become accustomed to the traps and minimize trap shyness/avoidance due to neophobia. We pre-baited the sampling areas with shredded coconut. Trapping commenced four days after the second bait application (10 days after the first application) for Treatment Areas 1 and 2 and two days after the second bait application (seven days after the first application) for Treatment Area 3. The decision to delay sampling for Treatment Areas 1 and 2 was made on site, based on perceived bait uptake rates by rats using daily bait monitoring observations, with the goal of assuring ample time for individuals to have access to bait prior to sampling. Sampling of Treatment Area 3 only two days after the second bait application was due to low persistence of bait (presumably due to high bait uptake by rats) based on daily bait monitoring observations.

We euthanized trapped rats and examined them for signs of fluorescence from the pyranine biomarker with UV lights. Examination included oral and anal orifices and gastrointestinal (GI) contents. Sex and body mass were also recorded.

Radio Tacking and Marking

To help better understand the movement of rats in our treatment areas, and to determine whether animals from outside of the treatment areas would relocate into the sampling areas, we used both tail-marking and radio telemetry techniques. We trapped and marked a total of 40 rats from habitat outside of each treatment area: 30 rats were tail-marked with a nontoxic felt-tip marker, and 10

rats were affixed with VHF radio collars (Holohil model BD-2C; Holohil Systems Ltd., Ontario, Canada). We marked rats from each site prior to each first bait broadcast and released them at the site of capture. Due to logistical complications, only eight rats were radio collared outside Treatment Area 1, and thus 32 rats were tail-marked. Due to logistical and time constraints, rat movement via radio telemetry was assessed opportunistically, when time allowed.

RESULTS

Treatment

During this study, we hand-broadcasted approximately 270 kg of nontoxic B-25W bait pellets within each treatment area. Due to time constraints and logistical complications (e.g., rough terrain, encounters with wasps), approximately 90% of Treatment Area 2 (forest) was baited on day 0, with the remaining area baited early the next morning. Although all four bait persistence plots were baited on day 0, the collection of monitoring data did not begin until day 1 of the first broadcast. The second application occurred on day six for Treatment Areas 1 and 2, and on day five for Treatment Area 3. Treatment Area 3 (SWAA) was baited on day five (the earliest a second application is allowed based on the product label) due to the extremely low levels of bait observed on the ground following the first application.

Bait Monitoring

We conducted bait monitoring each day, including the day of application. An exception to this was no monitoring data was collected on day 0 of the first broadcast for Treatment Area 2, due time constraints.

Bait Persistence

The average amount of initial bait measured within the four persistence plots were 30.3, 33.8, and 30.5 g for Treatment Areas 1, 2, and 3, respectively (measured on day 0 of the first application for Treatment Areas 1 and 3, and day 1 for Treatment Area 2). Daily bait persistence values, averaged by treatment area, are depicted in Figure 1. Bait persisted the longest on Plot 1 of Treatment Area 2, with bait disappearing completely on day 18 after the first broadcast. Bait disappeared the fastest from Plot 3 of Treatment Area 3, with bait disappearing completely on day 1 after the first broadcast and again day 0 after the second application. In the latter instance, all bait was gone by the time the persistence plot was monitored, less than an hour after the completion of the second application. Values with slight increases over time, not due to the addition of bait from the second application, are likely either due to the increased weight of the pellet after a rain event, or measurement error.

Differences in trends of bait persistence were observed among treatment areas, with bait persisting the shortest amount of time in Treatment Area 3 (SWAA) and persisting the longest in Treatment Area 1 (shrub/grassland). All treatment areas showed high rates of consumption.

Bait Condition

Within each treatment area, bait condition remained relatively constant, and was observed consistently and predominantly as "hard, intact, whole" (score of 1). Although rain events were relatively scarce throughout the study, some rainfall did occur; however, by the time the bait exposure plots were assessed, the bait typically appeared dry and intact. On occasion, bait following a rain event was recorded as "soft, intact, whole" (score of 3); however, in each case, all bait the following day returned to its original condition (score of 1). (Table 1)

Table 1. Scoring convention for monitoring of bait condition within bait exclusion plots.

Score	Criteria
1	Bait hard, intact, whole
2	Bait hard, intact, partially gone
3	Bait soft, intact, whole
4	Bait soft, intact, partially gone
5	Bait mushy, disintegrated
6	Bait dry, disintegrated
7	Bait gone

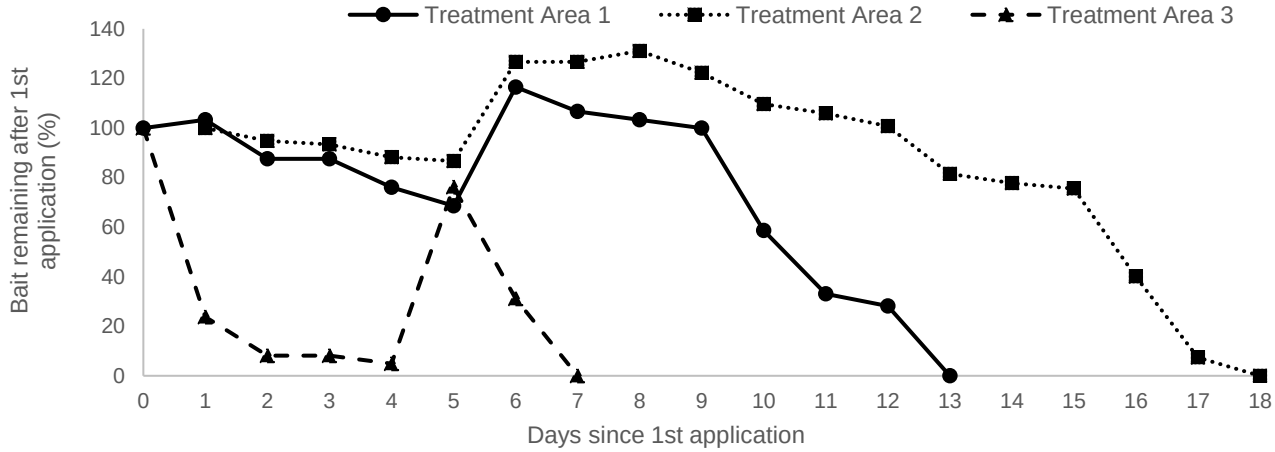

Figure 1. Persistence of bait over time for all three treatment areas (bait persistence plots). Values are the mean of all four persistence plots for each treatment area. Sharp increases occurring on day 5 or 6 indicate the day a 2nd application occurred.

Table 2. Timeline for bait applications and summary of results for 2017 placebo bait uptake trial on Wake Atoll. Gray cells indicate bait application days for corresponding treatment areas (TA).

TA #	Since 1st bait application (days)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	Since 2nd bait application (days)							0	1	2	3	4	5	6	7	8	9	10		
	Bait remaining after 1st app. (%)	100	103	88	88	76	69	117	107	103	100	59	33	28	0	0	0	0		
	Positive for biomarker (# of rats)												12	8	3	0	-	2		
	Negative for biomarker (# of rats)												0	0	0	0	-	4		
2	Since 2nd bait application (days)							0	1	2	3	4	5	6	7	8	9	10	11	12
	Bait remaining after 1st app. (%)	-	100	95	93	88	87	127	127	131	122	110	106	101	81	78	76	40	7	0
	Positive for biomarker (# of rats)												4	7	5	4	5	7		
	Negative for biomarker (# of rats)												0	0	0	0	0	0		
3	Since 2nd bait application (days)						0	1	2	3	4									
	Bait remaining after 1st app. (%)	100	24	8	8	5	76	31	0	0	0									
	Positive for biomarker (# of rats)								12	18	112									
	Negative for biomarker (# of rats)								0	0	6									

Bait Uptake Evaluation

We inspected a total of 209 rats for pyranine biomarker presence in this study, including 29, 32, and 148 rats from Treatment Areas 1, 2, and 3, respectively (Table 2). Of the total rats inspected, 122 were female and 87 were male. The mean body mass was 49 g, with the smallest and largest individuals at 21 and 72 g, respectively.

Treatment Area 1: This area was located in the northeast part of Wake Atoll and consisted of mixed shrub/grassland habitat. A total of 29 rats were sampled, including 18 females and 11 males, averaging 52.7 and 53.0 g, respectively. All rats sampled while bait was still on the ground tested positive for bait uptake. During the last sampling period, four days after bait was no longer detectable on the ground, four rats were captured with no signs of pyranine fluorescence. Additionally, the two rats that were identified as positive for biomarker on the same day showed very faint signs of pyranine fluorescence. With no more access to bait, it is reasonable to believe that pyranine had already been cleared from the digestive tract given the observations of Pitt et al. (2013) that demonstrate that pyranine is only an effective biomarker for three days after feeding.

Treatment Area 2: This area was located in the western part of Wake Atoll and consisted of closed-canopy forest vegetation. A total of 32 rats were sampled, including 16 females and 16 males, averaging 46.7 and 48.9 g, respectively. No rats were identified as negative for biomarker from the 32 individuals inspected; all rats sampled while bait was still on the ground tested positive for bait uptake.

Treatment Area 3: This area was located in the southern part of Wake Atoll and consisted of habitat surrounding the SWAA, characterized by high rat densities. A total of 148 rats were sampled, including 88 females and 60 males, averaging 47.5 and 50.6 g, respectively. As with the other treatment areas, we waited a few days after the second bait application to trap rats to allow ample time for rats to access bait; however, what we did not expect was for the bait to disappear so quickly. Although we attempted to move up the timing of sampling, we were unable to sample any rats while bait persisted on the ground (determined by the monitoring plots). Up to three days after the second application, all rats tested positive for biomarker; however, on day four (the third day with no bait observed on the ground), approximately 5% (6/118) of rats sampled were negative. Of these six negative individuals, four were female and two were male. Of the 112 positive rats sampled from the same day as the negative individuals, the intensity of pyranine fluorescence varied, from bright to very faint.

In total, 98% (195/199) of the rats that were scored as positive for pyranine biomarker showed fluorescence in the GI contents, whereas results based on mouth and anus inspection were less consistent.

Rat Movement

We typically conducted telemetry in either the morning or afternoon, and not at night. Rats were observed to be quite active throughout the day at all three treatment areas, especially Treatment Area 3, where on numerous occasions collared rats were moving while being located. Observed movement varied by individual and day, with some rats consistently located in the vicinity of their site of release and others observed to have moved considerable distances. Not all individuals were located during each attempt.

Treatment Area 1: Rats were often located in close proximity to initial site of release (within 50 m), with occasional instances of rats having travelled up to 100 m. No collared rats were tracked moving into the sampling area. Additionally, no marked individuals were trapped within the sampling area during the bait uptake evaluations. One collared rat was found dead from unknown cause two days after the first application.

Treatment Area 2: Although no rats were observed entering the sampling area, rats were often located 50-100 m from their initial location and not in any consistent direction from the site of release. One individual was observed to have travelled approximately 200 m.

Treatment Area 3: Documented rat locations for all 10 rats are presented in Niebuhr et al. 2018. Rats were regularly observed 50-150 m from the site of release, with three individuals observed traveling >300 m. Often these individuals were found to have moved within the sampling area. On numerous occasions some individuals that were

previously located in the sampling area had travelled back to the release site the following day, while others appeared not to have wandered far. It is not known if these latter individuals had also travelled longer distances and returned prior to us locating them. Although difficult to determine, based on our observations rat movements did not appear to show any consistent daily migration into and out of the SWAA. During the bait uptake evaluations, two female collared rats were caught in the sampling area (approximately 250 m from the collaring and release site). Both rats were positive for pyranine.

DISCUSSION

Question 1

Is the label application rate for the selected bait type high enough to provide every rat within the project boundary with an opportunity to consume a lethal amount of bait? Based on our findings, the prescribed maximum bait application rate for B-25W (18 kg/ha followed by 9 kg/ha) was not high enough to provide every rat an opportunity to consume enough bait at all treatment areas. While this application rate was high enough for Treatment Areas 1 and 2, it was not sufficient for the SWAA area, Treatment Area 3. Note, although four rats in Treatment Area 1 were observed negative for pyranine, all four occurred on the fourth day of no bait observed on the ground (day 10 after the second application). Only two rats were observed positive for pyranine on the same day, both reported as being very faint. Pyranine is considered a good short-term marker for rats but has failed to be retained reliably past three days (Pitt et al. 2013). Therefore, we do not consider these negative observations from Treatment Area 1 to be a result of an inadequate application rate, but rather a result of the timing of the sampling. All rats sampled in Treatment Area 2 showed evidence of pyranine exposure.

The six negative rats from Treatment Area 3 were sampled on the third day of no bait on the ground (day 4 after the second application). On the same day, 112 rats were observed as positive for pyranine, the majority of which displayed bright fluorescence. We recognize that these observations were made from a nontoxic bait study; it is possible that rats that had ingested enough bait early on would normally have been removed from the population in a real bait application scenario, but instead were allowed to remain and continue to compete for bait. This could lead to consumption rates that are much higher than those that would be observed during an actual eradication attempt. Our radio telemetry data also documented that it is possible that the negative rats might have recently moved in from beyond the extent of our treatment area. However, due to the extremely high rate of bait disappearance attributed to rat engagement (e.g., high percentage of biomarker positive rats, visual observations of rats eating bait seconds after being applied), combined with negative biomarker rats observed, we conclude the overall rate of application was insufficient for the SWAA. For any future eradication actions at Wake Atoll, we recommend that alternative strategies, bait application rates, bait types, or changes in duration between bait applications be considered for use in the SWAA, to confidently provide all rats access to sufficient bait.

Question 2

Will all rats within the project boundary consume bait despite access to natural and commensal food sources available at the time of the study? Within all three treatment areas, rats appeared to readily consume bait, despite presumed access to natural and anthropogenic food sources. Bait condition throughout the study remained good, even following infrequent rain events. In Treatment Areas 1 and 2, all rats sampled while bait remained on the ground showed signs of biomarker exposure. While rat numbers within Treatment Area 3 were observed to be extremely high, likely due to presumably greater than natural availability of anthropogenic food subsidies within the SWAA, the bait was consumed at an extremely high rate. Because rat sampling at the SWAA only occurred days after the last bait was consumed, we cannot be certain whether negative rats had been exposed to the bait but rather chose to consume alternative foods available within the area. We recommend that preference for anthropogenic food items over rodenticide bait pellets be explicitly tested at the SWAA and other commensal habitats to determine whether increased bait applications will be sufficient or if alternative baits will be required.

We only observed movement of rats from outside of the treatment area into the sampling area in Treatment Area 3. Here, multiple rats were observed travelling more than 300 m, with some individuals remaining in the sampling area for multiple consecutive days. Therefore, it is possible that rats from outside the treatment area travelled into the sampling area and were trapped and inspected, but they would have had to not consume any bait along the way. Indeed, two collared rats that had moved from outside the treatment area and into the sampling area were inspected and found to be positive for the biomarker. Additionally, while we did observe evidence of crabs interacting with bait within all treatment areas, based on our bait uptake results (203 of 209 rats positive for pyranine), it is reasonable to assume crabs were not, at least initially, denying rats access to bait on the ground; however, a better understanding of the role of crabs in bait disappearance during an eradication event is recommended.

Question 3

How fast do rodenticides disappear when applied to differing habitat types on Wake Atoll? The rate of bait disappearance differed by site (Figure 1). Overall, bait on the ground disappeared the slowest within Treatment Area 2 (12 days after the second application), followed by Treatment Area 1 (seven days after the second application), and disappeared the fastest within Treatment Area 3 (two days after the second application). Some monitoring plots within Treatment Area 3 showed bait disappearance after one day, with one instance of bait disappearing within hours of the application.

In summary, we evaluated the adequacy of the maximum label-prescribed bait application rate for B-25W at three sites on Wake Atoll. Our conclusion is that the area around and including the SWAA would require supplementary effort. Our results support the assertion of Brown et al. (2013) that the SWAA area is a "high risk" habitat, and that areas with high alternative food abundance may have contributed to the 2012 eradication failure. During the

prior eradication attempt, additional bait swaths were flown over the SWAA for a reported bait application rate of >27 kg/ha during the first application and 9-18 kg/ha during the second application (Figures 3 and 4 in Island Conservation 2013). Given the rapid disappearance of bait from this area and the regular immigration of rats from distant habitat in our study, we recommend that an even greater application rate be prescribed and that the heavier treatment be extended over a much larger area surrounding the SWAA. We further suggest that additional effort is needed to confirm palatability of rodenticide pellets for rodents accustomed to anthropogenic food sources in the SWAA and commensal areas, to determine whether supplementation with alternative baits may be required for a successful future eradication.

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