Monitoring Brown Treesnake Activity Before and After an Automated Aerial Toxicant Treatment

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Executive Summary

During the first *in situ* evaluation of an automated aerial bait delivery system for control of invasive Brown Treesnakes (*Boiga irregularis*), acetaminophen-laced baits were applied at approximately 120/ha over a 110-ha forested test plot on the Pacific Island of Guam. To evaluate the suppressive effect of this bait application, we monitored the rates at which nontoxic dead newborn mouse baits were removed from bait stations as an index of relative snake abundance. We evaluated "bait take rates" before and after bait application, in the treatment plot and in surrounding untreated habitat, for a before-after-control-impact (BACI) experimental design. A total of 4,420 georeferenced baits were distributed in random transects from one month before until nearly one year after the bait application, allowing temporal analysis of the suppressive effect and spatial analysis of reinvasion of the treatment plot. The average take rate in the treated plot for the first 30 days after the toxic bait application was 41.2% lower than the pre-application rates, and there was no decrease in the surrounding untreated habitat. A suppression effect was still evident nearly a year after the bait application. Reinvasion across a treatment boundary that was within contiguous forest habitat appeared faster than across boundaries formed by narrow roads, indicating a temporary partial barrier effect of roads. Of a subset of baits monitored by camera, few were taken by nontarget species. There was no evidence of an increase in rodent abundance following this limited suppression of their primary predator. Our results suggest that automated aerial bait applications can have a suppressive effect on Brown Treesnake abundance. We anticipate that repeated and sustained applications could achieve and maintain drastically reduced Brown Treesnake numbers on a landscape scale, potentially improving biosecurity and enabling experimental reintroduction of native birds extir-

pated by Brown Treesnake predation.

Introduction

Aerial delivery of baits for wildlife management has enabled landscape-scale implementation of programs for disease prevention, reduction in damages caused by invasive wildlife, and restoration of native flora, fauna, and ecosystem functions. Programs for the vaccination of wild carnivores via aerial baiting have been remarkably successful at reducing rabies transmission in North America and Europe (Rupprecht et al. 2004, Slate et al. 2009, Freuling et al. 2013), and aerial baiting has been proposed as a tool to prevent spread of bovine tuberculosis by invasive brushtail possums (*Trichosurus vulpecula*) in New Zealand (Nugent et al. 2016). Aerial sowing of toxic baits for the lethal control of injurious species has been employed to target brushtail possums (Eason et al. 1993, Henderson et al. 1999), foxes and dogs (*Vulpes vulpes*, *Canis lupus familiaris*, and *C. l. dingo*; Fleming et al. 2006), feral cats (*Felis catus*; Algar et al. 2002), red deer (*Cervus elaphus*; Fraser and Sweetapple 2000), brushtailed rock wallaby (*Petrogale penicillata*; Mowbray 2002), European rabbits (*Oryctolagus cuniculus*; Shaw et al. 2011, Terauds et al. 2014), and various invasive ants (Boland et al. 2011, Hoffman et al. 2016). Aerial application of toxic bait has been particularly successful for the eradication of rats and mice from island ecosystems (Howald et al. 2007, Russell and Holmes 2015), resulting in substantial conservation gains (LeCorre et al. 2015, Jones et al. 2016) and ecological effects including enhanced coral reef productivity (Graham et al. 2018).

The accidental introduction of the Brown Treesnake (*Boiga irregularis*) to the Pacific island of Guam caused severe economic and ecological damage. The Brown Treesnake invasion front coincided with a wave of precipitous declines in bird diversity and abundance (Savidge 1987, Wiles et al. 2003) and resulted in the extirpation of eleven of Guam's native forest birds and the extinction of the Guam flycather (*Myiagra freycineti*) and the Guam subspecies of bridled white-eye (*Zosterops conspicillatus conspicillatus*) and rufous fantail (*Rhipodura rufifrons uraniae*; Savidge 1987, Wiles et al. 2003). The Guam rail (*Gallirallus owstoni*) and the Guam Micronesian kingfisher (*Todiramphus cinnamominus*) are extinct in the wild, though captive populations have been maintained in the hope of reintroduction following Brown Treesnake suppression actions. This loss of an entire forest avifauna has resulted in cascading ecological consequences including disturbance of seed dispersal (Rogers 2011), plant reproduction and recruitment (Mortensen et al. 2008, Rogers et al. 2017), forest regeneration (Perry and Morton 1999), and arthropod suppression (Rogers et al. 2012). Predation by Brown Treesnakes has negatively impacted nearly all native vertebrate populations on Guam (Wiles 1987, Rodda and Fritts 1992, Fritts and Rodda 1998) as well as non-native and domestic animals (Fritts and McCoid 1991, Wiewel et al. 2009). In addition to these ecological impacts, other detriments of the Brown Treesnake invasion of Guam include damage to electrical power infrastructure, predation on domestic animals, human envenomations, higher costs of shipping from Guam, and threats to the tourism industry (Rodda and Savidge 2007).

An array of tools and strategies have been devised, evaluated, and implemented to prevent the spread of Brown Treesnakes to other snake-free islands and to reduce snake-caused damages around focal resources (Clark et al. 2018, Engeman et al. 2018). Following the apparent success of these interdiction measures, the multi-agency Brown Treesnake Technical Working Group (2015) has identified landscape-scale suppression of Brown Treesnakes as a key objective, to enable reintroduction of native species and recovery of natural habitats.

Over the last two decades, the US Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Wildlife Services (WS) National Wildlife Research Center (NWRC) has developed technologies that have advanced landscape-scale suppression of Brown Treesnakes from a remote aspiration toward a practical reality. Dead newborn mice (DNM) were evaluated as the most effective bait for Brown Treesnakes (Shivik and Clark 1997). Acetaminophen was identified as a safe and humane oral toxicant, with an 80-mg dose proving 100% lethal within 24 to 48 hours of ingestion in cage trials (Savarie et al. 2000). Placement of acetaminophen-treated DNM baits in plastic tube bait stations demonstrated that Brown Treesnake abundance can be suppressed on a landscape scale (Savarie et al. 2001) and can serve as a cost-effective alternative to trapping (Clark et al. 2012). Because ground-based bait applications are limited to easily accessible areas that are adjacent to roads and trails, aerial delivery of baits will be required for cost-effective treatments of large and remote areas. Further NWRC studies established that aerially-delivered baits equipped with "flotation devices" can be suspended from the forest canopy where they are accessible to arboreally-foraging Brown Treesnakes and less accessible to terrestrial nontarget species such as ants, crabs, and rodents (Shivik et al. 2002, Savarie & Tope 2004, Savarie et al. 2007), and that aerial bait applications can significantly suppress Brown Treesnake abundance (Shivik et al. 2002, Clark and Savarie 2012, Dorr et al. 2016). In these studies, baits were manually prepared by inserting acetaminophen tablets into the DNM via the oral cavity, manually affixing the DNM bait to a flotation device, and hand-broadcasting baits from a helicopter. Although these studies established proof of concept for Brown Treesnake suppression by aerial baiting, Dorr et al. (2016) concluded that the labor demands to manually prepare and hand-broadcast baits would not be practical or cost-effective for operational use on a large scale.

With funding from the U.S. Department of the Interior, Office of Insular Affairs, NWRC partnered with a private engineering firm, Applied Design Corporation (Boulder, Colorado) to automate bait production and distribution. The resulting automated bait manufacturing system (ABMS) adheres an acetaminophen tablet to a DNM bait with hot-melt glue, then glues the DNM into a formed pulp-paper capsule. The capsule is folded around the bait and wrapped with a length of cornstarch ribbon, which is glued to the capsule on one end and the endcap of an outer cardboard tube on the other end. The ribbon-wound capsule is then tucked into the outer tube, comprising a complete "bait cartridge," similar in size and shape to a roll of U.S. quarters. The entire cartridge is biodegradable. As cartridges are produced, they are fed into a plastic carton holding 900 cartridges for later distribution by an automated delivery system (ADS) module mounted in a helicopter. Each carton fills one magazine, and four magazines are loaded into the ADS. In flight, bait cartridges from each magazine feed into an ejector port where they are forcibly fired into the air. On ejection, the inner bait capsule slides out of the outer tube, exposing the bait and the ribbon which are still attached to the outer tube. Upon landing on the forest canopy, this bait/ribbon/tube assembly tangles in the treetops where Brown Treesnakes can feed on the baits. This system can dispense baits at a rate of four per second. At a bait density of 120/ha, 30 ha of forest can be treated with a payload of 3,600 baits within 15 minutes of firing time. This system earned USDA Wildlife Services the Federal Laboratory Consortium's (FLC) Award for Excellence in Technology Transfer and the FLC Award for Notable Technology in 2015.

Siers et al. (2017a, in press) conducted the first *in situ* evaluation of this system over 110 hectares of secondary forest on Guam in July of 2016. The study design called for two bait applications at the maximum approved rate of 120 bait per hectare. Jams of the ADS ejection mechanisms and failures of cartridges to properly open in flight were frequent due to wind forces and internal friction in bait cartridge components. Performance was improved by on-site remedial engineering, but based on counts from helicopter-mounted video recorders it is presumed that no more than 50% of the bait cartridges were opened so that baits were available for consumption. Therefore, the results were considered to be indicative of only a single effective application at 120 baits per hectare, totaling no more than 13,200 baits available to Brown Treesnakes. Fifty-one of the baits were equipped with VHF radio transmitters inserted into the DNM body cavity. Of these, only three (5.9%) were confirmed to have been ingested by Brown Treesnakes. Assuming a moderate density of 25 Brown Treesnake per hectare (a conservative estimate based on Rodda et al. 1999), 2,750 snakes would have been exposed to baits; if 5.9% of the 13,200 baits were consumed by Brown Treesnakes, approximately 779 (28%) of the snakes in the treatment area would have taken a bait. Several more baits appeared to have been ingested and regurgitated by snakes, though this could not be confirmed. Snakes in cage trials that had regurgitated baits after acetaminophen intoxication later died (Savarie et al. 2000, Johnston et al. 2002, Nafus and Siers 2018), so the actual mortality rate based on this index could be higher.

Here we report on rates at which unadulterated DNM baits were taken from bait stations as an additional index of snake suppression following the 2016 automated aerial bait application reported in Siers et al. (2017a, in press). These "bait take rates" were monitored for more than one month prior to bait application and for nearly one year after, both in the treatment area and surrounding untreated habitat. Our objectives were to: 1) evaluate the degree and duration of a suppressive effect of the automated aerial bait application, 2) explore spatial aspects of snake suppression and recovery in and around the treatment area including reinvasion across treatment boundaries formed by roads versus contiguous forest. Because Brown Treesnakes are known to suppress rodent abundance in forest habitats (Wiewel et al. 2009) and it is anticipated that rodent abundance may increase as a result of snake suppression (Dorr et al. 2016), an additional objective was to concurrently monitor changes in rodent abundance following this bait treatment.

Methods

Study Site

The evaluation of the automated aerial bait delivery system (ADS) took place in July of 2016 over 110 ha of degraded secondary forest on the Marbo Annex of Andersen Air Force Base (Figure 1) in Yigo, Guam, at approximately 13,508°N, 144.873°E (Siers et al. 2017a, in press). This area had no previous Brown Treesnake or rodent control activities, with the exception of snake trapping along the perimeter fence around the power substation on the northern border of the treatment area.

Snake activity monitoring

We monitored the removal of unadulterated (nontoxic) DNM baits as an index of Brown Treesnake foraging activity and a proxy for snake abundance, as employed by Clark and Savarie (2012), Sugihara et al. (2015), and Dorr et al. (2016). Savarie et al. (2001) validated that decreases in toxic DNM bait take rates coincided with decreases in Brown Treesnake abundance and survival estimates from capture-mark-recapture models. Frozen DNM were provided by USDA Wildlife Services, purchased from a known supplier (Noble Supply and Logistics, Honolulu, Hawaii). Baits were offered in 30-cm lengths of 5.1-cm diameter polyvinyl chloride (PVC) tube, or "bait tubes," suspended by nylon cord from surrounding vegetation approximately 1.5 m above ground level (Figure 2). Bait tubes are intended to prevent nontarget species, such as crabs, rats, and monitor lizards (*Varanus indicus*), from taking baits. A ¼" bolt is passed through each end of the tube to further reduce access by nontargets. To evaluate the proportion of baits taken by nontarget species, a subset of the bait tubes were monitored with an infrared game camera triggered by removal of the DNM from a pressure-sensitive switch (as employed by Sugihara et al. 2015, Abernethy et al. 2016, and Siers et al. 2018). Identity of the species removing the bait was confirmed by reviewing the camera images.

Each transect of bait tubes was composed of 10 tubes spaced at least 20 m apart. We assigned transect origin points by generating a list of random GPS coordinates within the treatment area or within a 225-m buffer of untreated reference habitat surrounding the treatment plot (Figure 3). We placed the first bait tube at the origin coordinates, then followed a randomly-generated bearing for the remaining locations, deflecting at a 90-degree angle when encountering a plot boundary. We recorded GPS coordinates for each bait tube location. We placed a single nontoxic DNM in each bait tube, and returned 48 hours later to record the presence or absence of the bait. Bait tubes were then removed for repositioning at another randomized transect location.

We monitored baits in the treatment and reference habitats for 5 weeks before and 48 weeks after aerial bait application (Table 1). *Post hoc*, we divided the post-baiting monitoring period into four quarters for evaluation of temporal trends in bait take rates.

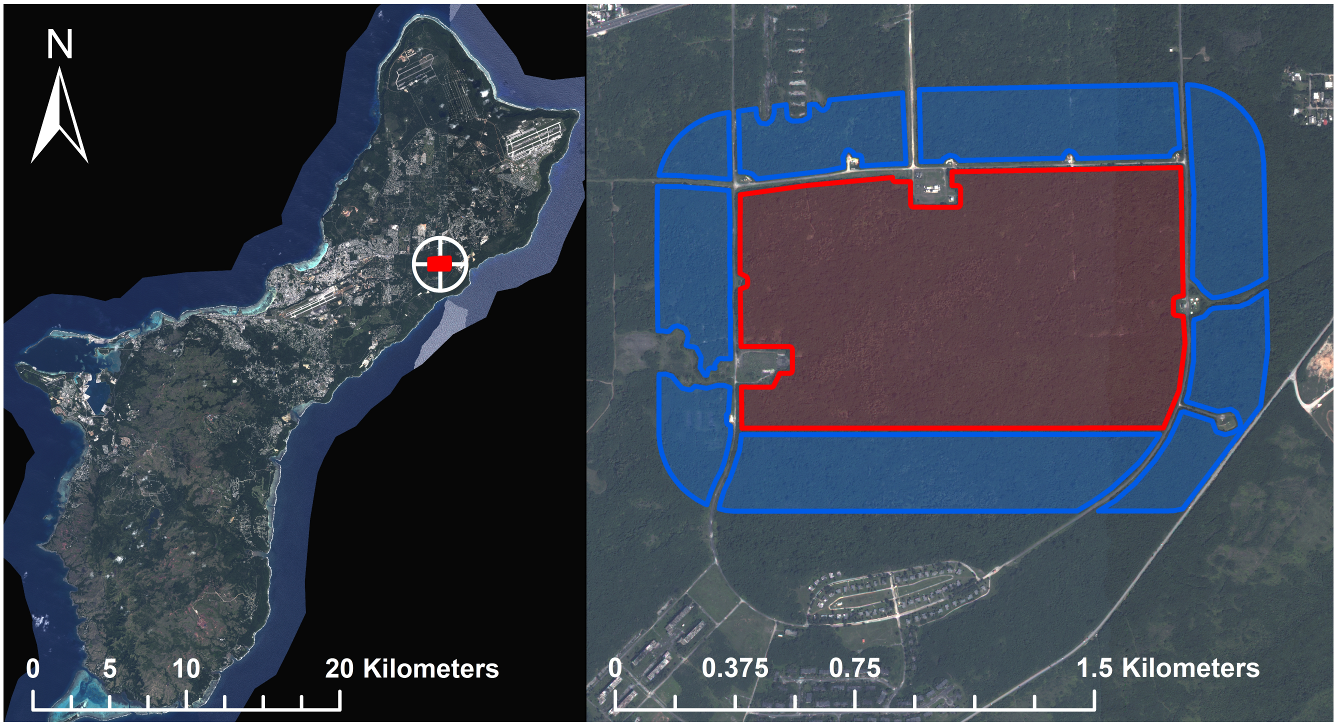

Figure 1: Left: location of the test site on Guam (crosshairs). Right: 110-hectare treatment area (red polygon) surrounded by untreated reference habitat (blue).

Table 1: Date ranges of monitoring periods, with sample sizes (N) of baits offered in the treatment area (TRT) and surrounding untreated reference habitat (REF).

Date Range	Period	Days	TRT	REF	TOTAL
6 Jun 2016 – 12 Jul 2016	Before	37	370	370	740
8 Aug 2016 – 31 Oct 2016	Q1	85	510	490	1,000
1 Nov 2016 – 31 Jan 2017	Q2	92	540	560	1,100
1 Feb 2017 – 30 Apr 2017	Q3	89	395	385	780
1 May 2017 – 10 Jul 2017	Q4	71	405	395	800
	TOTAL	374	2,210	2,210	4,420

Temporal analysis

We graphically represented the effect of bait application by plotting bait take rates over continuous time for the treatment and reference areas. Because binomial bait take data are inherently noisy, we smoothed the response variable by averaging bait take rates over a 90-day moving window and plotted the 95% asymptotic binomial confidence intervals for the estimated mean.

We tested significance of the treatment effect in a binomial generalized linear mixed effects model with a before-after-control-impact (BACI) experimental design. Bait status (taken/not taken) was the binary response variable ("[1,0]"). The fixed effects of interest were time period ('before' or 'after' aerial bait application), represented as a "BA" categorical covariate in the model, and treatment status of the location (untreated 'control' habitat or treated 'impact' site), as "CI". The statistical significance of the impact is inferred from the significance of a "BA*CI" interaction term included in the model. We specified the basic fixed-effects model as:

$$[1,0] \sim BA + CI + (BA * CI)$$

Because we do not consider bait tubes within transects statistically independent, and bait take rates within the same time period tend to covary across closely-associated plots, we also considered

Figure 2: Example of a PVC "bait tube" suspended from vegetation. Note the bolts passing through each end of the tube to exclude larger nontarget organisms such as crabs.

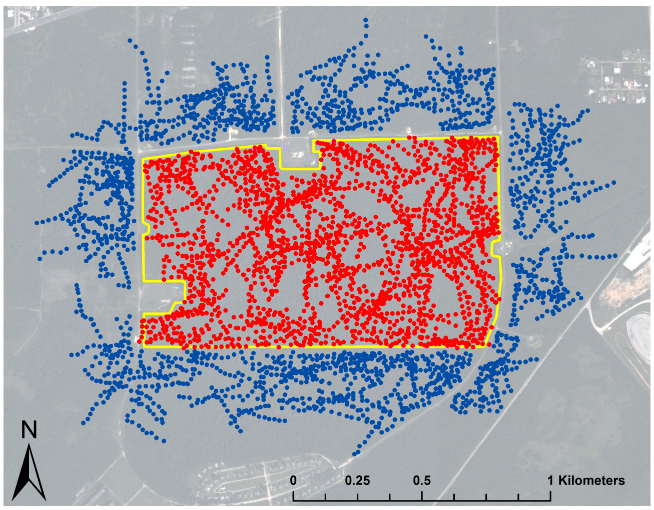

Figure 3: Randomized bait tube transect locations in the treatment area (red) and surrounding untreated reference habitat (blue). We monitored 2,210 baits in each.

the inclusion of "Transect" and "Week" as random effects in candidate models to account for spatial and temporal non-independence. Using the "(1 | X)" notation for command 'glmer' in the 'lme4' package, the fully specified model including both random effects would be:

$$[1,0] \sim BA + CI + (BA * CI) + (1 \mid Transect) + (1|Week)$$

Models with and without these random effects were compared, with the best model chosen based on the lowest AIC_C value.

We evaluated the initial impact of treatment by estimating the BA*CI term for a subset of the data including only the bait take rates from the 37 days before treatment and the first 30 days post-treatment. We then assessed the persistence of a treatment effect by subsequently running separate models including data from the pre-treatment period and each of the four post-treatment quarters detailed in Table 1. An overall reduction in bait take rates throughout the study site during the last quarter of sampling, irrespective of treatment, was evaluated by a model containing only the "BA" term and random effects. For all tests, we set statistical significance at $\alpha = 0.05$.

Spatial evaluation

To investigate spatial patterns in snake suppression and reinvasion from surrounding habitats, the distance of each bait stations from the treatment boundary was calculated in ArcGIS. With the treatment boundary at 0 m, distance into the core of the treatment area was represented with positive values, and distance away from the boundary into the surrounding reference habitat assigned negative values. To compare changes in bait take rates across a road boundary with those across contiguous forest, data were subset as depicted in Figure 4, with 'road' or 'forest' factor levels recorded as point attributes.

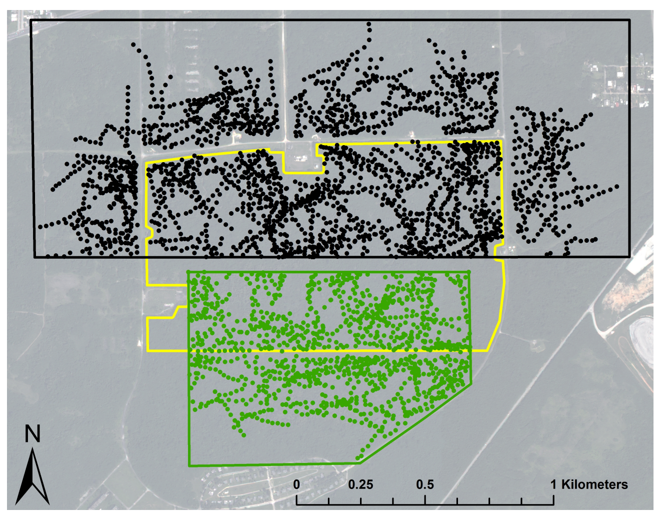

Figure 4: Subsets of bait tubes included in the spatial evaluation of snake suppression and reinvasion into treatment areas bordered by roads (black) or within contiguous forest (green).

We graphically depicted variation in bait take rates from the core of the treatment area to the outer extent of the reference habitat for each of the boundary types, with 100-m moving averages and 80% confidence limits for those averages. We then predicted distance effects crossing each of the boundary and treatment factors with separate fixed effects logistic regressions and plotted response curves with estimation envelopes (±1 standard error) for visual interpretation.

Rodent monitoring

Once each quarter following bait applications, we established 8 random transects of 10 Haguruma cage traps spaced at least 20 m apart in each of the treatment and reference habitats to evaluate captures per unit effort as an index of change in rodent abundance following Brown Treesnake suppression. We recorded traps that were closed when checked but did not contain rodents (were empty or contained nontarget species) as nonfunctional and subtracted them from the level of effort. Due to time constraints, rodent activity was not evaluated prior to Brown Treesnake bait applications.

All animal use was reviewed and approved by the National Wildlife Research Center Institutional Animal Care and Use Committee, under Protocol QA-2621.

Results

Bait take rates were nearly identical between the treatment and control sites prior to bait application (see graphical representation of the timeline in Figure 5). Immediately following treatment, there was an obvious decrease in the bait take rate within the treated site, while the rate in the surrounding untreated habitat remained consistent with pre-treatment levels. Bait take rates continued to be lower in the treated area throughout the following year, though nearing convergence, and with overlapping confidence intervals, toward the end of the monitoring period.

Our fixed-effects BACI logistic regression model, based on the first 30 days of bait tube monitoring following toxic bait applications, indicated a highly significant impact via the BA*CI interaction term ($z = 3.887$, $p < 0.001$). The mean bait take rate within the treatment area (0.333) was 41.2% lower than in the surrounding untreated reference habitat (0.567). Comparing this fixed-effects model to mixed-effects models including random terms for week and transect, all candidate models outperformed the fixed-effects model based on AIC_C values, with the top model including both random effects and outperforming the fixed-effects model by 48.6 AIC_C units (Table 2).

In this top model, the BA*CI interaction (impact) term continued to be significant ($z = 3.014$, $p = 0.003$). Estimates of variance for both random effects were greater than zero (transect = 0.474, week = 0.154), though ± 1 standard deviation intervals would overlap zero (SD = 0.689 and 0.393). The mixed-effects model with both treatment and week random terms was used for all subsequent tests.

Models assessing the duration of treatment effect (significance of the BA*CI interaction term) over the following year, as subset into quarters (Figure 6), indicated a reduced effect size and diminished significance over time. By the final months of monitoring, the bait take rate in the treated area was still 15.73% lower than within the reference habitat, though the effect was no longer statistically significant ($z = 1.443$, $p = 0.149$). Comparing bait take rates throughout the entire study site before treatment and at the end of the monitoring period (Q4), irrespective of treatment, bait take was 17.13% lower after the treatment than before (0.498 versus 0.601, $p = 0.034$).

Spatial evaluation

Prior to bait application, the spatial distribution of bait takes with respect to the treatment boundary was relatively uniform, particularly across the boundary formed by roads ('Before' in Figure 7). During the first quarter after bait application, the depression of bait takes across the treatment plot boundary formed by contiguous forest was relatively uniform, while the difference across the road boundary was clearly more disjunct. This effect was even more pronounced during the following quarter. In the third and fourth quarters, differences across treatment boundaries became progressively less distinct, and bait take rates became more uniform across both boundaries and throughout the treatment and reference habitats. These response plots are offered for their heuristic value, and do not constitute a rigorous statistical test.

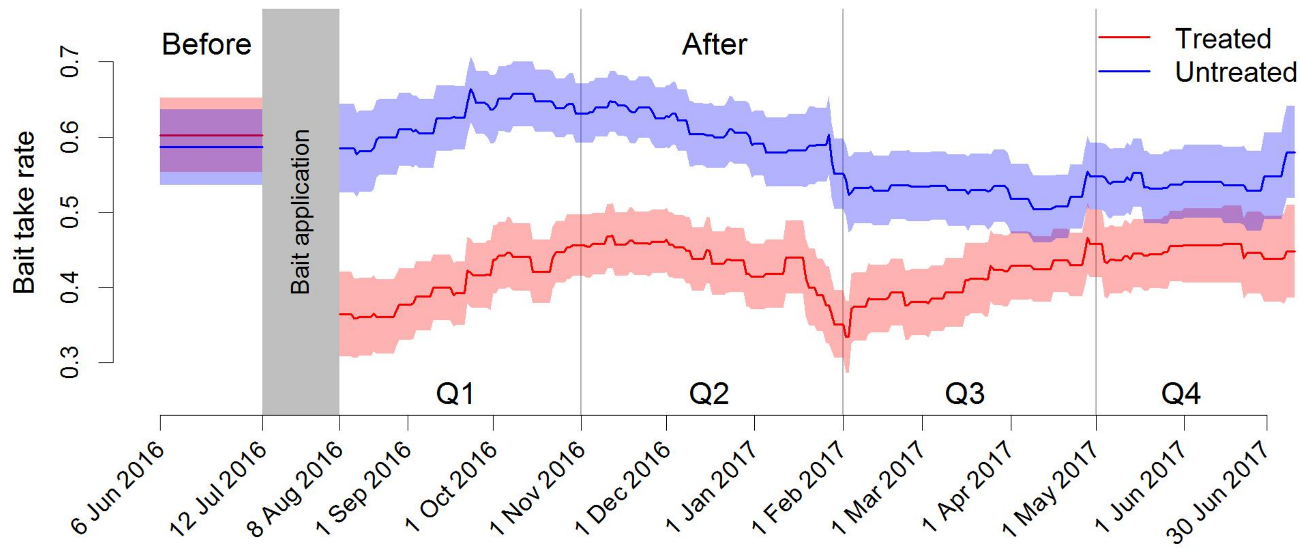

Figure 5: Chronology of bait take rates in treated and untreated habitat before and after aerial bait application. Bait take rate estimates are 90-day moving averages with shaded areas representing 95% binomial confidence intervals. Q1–Q4 represent the quarters for which subsequent statistical tests were applied.

Table 2: Model comparisons of fixed-effects and mixed-effects models. K = parameter count; AIC_C Aikake's Information Criteria corrected for small sample size; ΔAIC_C is the difference in AIC_C from the top model; LL = log-likelihood.

Model specification	K	AIC_C	ΔAIC_C	LL
BA+CI+(BA*CI)+(1 | Transect)+(1 | Week)	6	1433.9	0.00	-710.90
BA+CI+(BA*CI)+(1 | Transect)	5	1439.8	5.98	-714.90
BA+CI+(BA*CI)+(1 | Week)	5	1458.6	24.75	-724.90
BA+CI+(BA*CI)	4	1482.5	48.64	-737.24

Nontarget bait takes

Only eight bait tubes were monitored with cameras prior to the bait applications. Results from the four treatment area tubes were identical to the four from the reference area, both having one bait taken by a Brown Treesnake and one by an unknown organism (camera failed to trigger). From 9 August 2016 to 20 March 2017, we successfully observed 58 bait takes in the treatment area, of which 56 were by Brown Treesnakes and 2 were by monitor lizards. Of 62 takes in the reference habitat, all were by snakes with the exception of 1 by a monitor lizard. No baits were observed to have been taken by any other nontarget species. The overall rate of nontarget bait takes was 0.025 (95% CI = 0.0052-0.0713). With only three nontarget bait takes recorded, rates were too low for meaningful statistical tests of treatment or time effects.

Rodent monitoring

In 1,058 functional trap nights within the treatment area, we captured only one rat, during the first quarter post-baiting. Within the reference habitat, we captured only one rat in 1,068 trap nights, during the second quarter. Both rats were presumed to be *Rattus diardii* (per Wiewel et al. 2009). Rat captures were too low for meaningful statistical tests.

Discussion

Our monitoring of the disappearance of nontoxic baits following an automated aerial toxic bait application (Siers et al. 2017a, in press), reflects

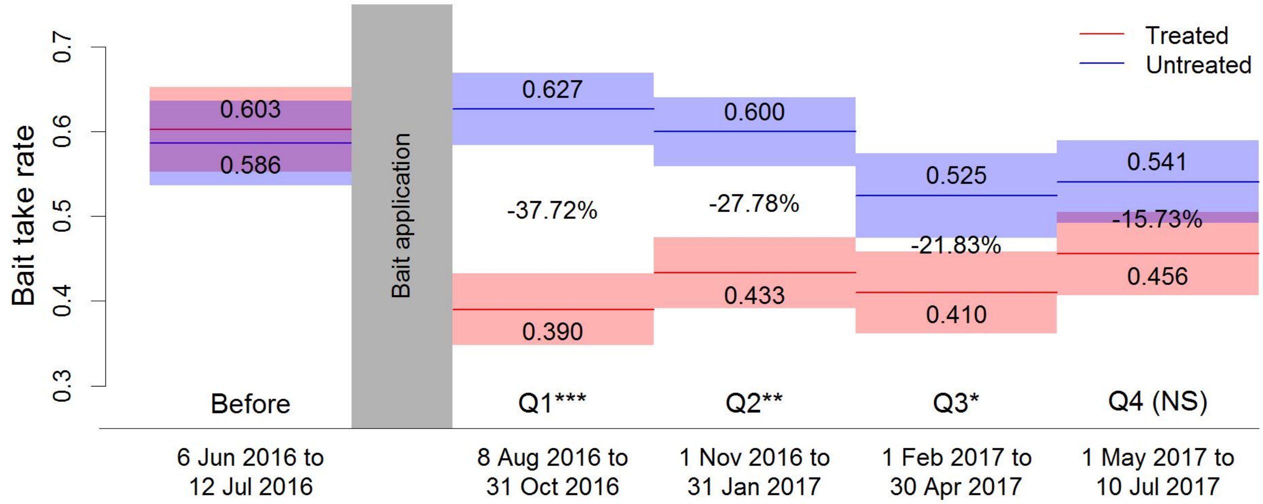

Figure 6: Comparisons of bait take rates from the pre-treatment period with post-treatment data pooled into quarters of approximately three months. P-values indicate significance of the BA*CI interaction (impact) term when comparing each respective quarter to the pre-treatment data: *** < 0.001; ** < 0.01; * < 0.05; NS > 0.05. Shaded areas indicate the 95% binomial confidence intervals for the respective bait take rates.

a distinct depression of Brown Treesnake foraging activity. To the extent that bait take rates can be interpreted as an index of relative abundance, these results indicate that automated aerial bait applications are effective at reducing Brown Treesnake numbers on a landscape scale. As described by Siers et al. (*ibid.*), the number of baits believed to have been available to snakes during this evaluation was likely equal to, or less than, one complete treatment at the EPA-approved maximum application rate of 120 baits per hectare. Bait take rates were immediately reduced by >40%, and the effect was evident for nearly a year after the treatment despite there being no significant barriers to reinvasion from neighboring habitat. In areas where drastic and sustained snake suppression is required, baits may be applied at 120/ha for up to nine treatments per year (according to the EPA pesticide label). Repeated and sustained bait applications might have the potential to maintain very low snake abundance as long as treatment continues.

The single treatment of the 110-ha plot appears to have had a spillover effect, as evidenced by the apparent migration of snakes from the reference habitat across the treatment boundaries into the snake-suppressed treatment zone, and the overall lower rate of bait takes across both treated and untreated habitat nearly a year after treatment. Caution should be taken in this latter interpretation, however, because our study did not include a remote untreated site to act as an external control for this particular test.

In any evaluation of the efficacy of a control tool, it is preferable to have multiple independent metrics. If we assume that the 41.2% decrease in bait take rates for the first 30 days after an aerial bait application is a direct and reliable metric of efficacy, such that we assume that 41.2% of the snakes in the 110-ha treatment area at the time of bait applications were killed, and that 25 Brown Treesnakes per hectare is a reasonable assumption of density (Rodda et al. 1999), we would estimate that this bait application killed 1,133 of 2,750 snakes. Based on the percentage of baits containing radio transmitters taken by snakes during this same treatment (Siers et al. 2017a, in press), we estimate that 779 of 2,750 snakes, or 28%, would have died after taking a bait. If only one of the baits containing transmitters found on the ground had been regurgitated by a lethally-intoxicated snake, the estimated overall mortality would be 1,035 snakes, or 38%. We consider the results of these two methods to

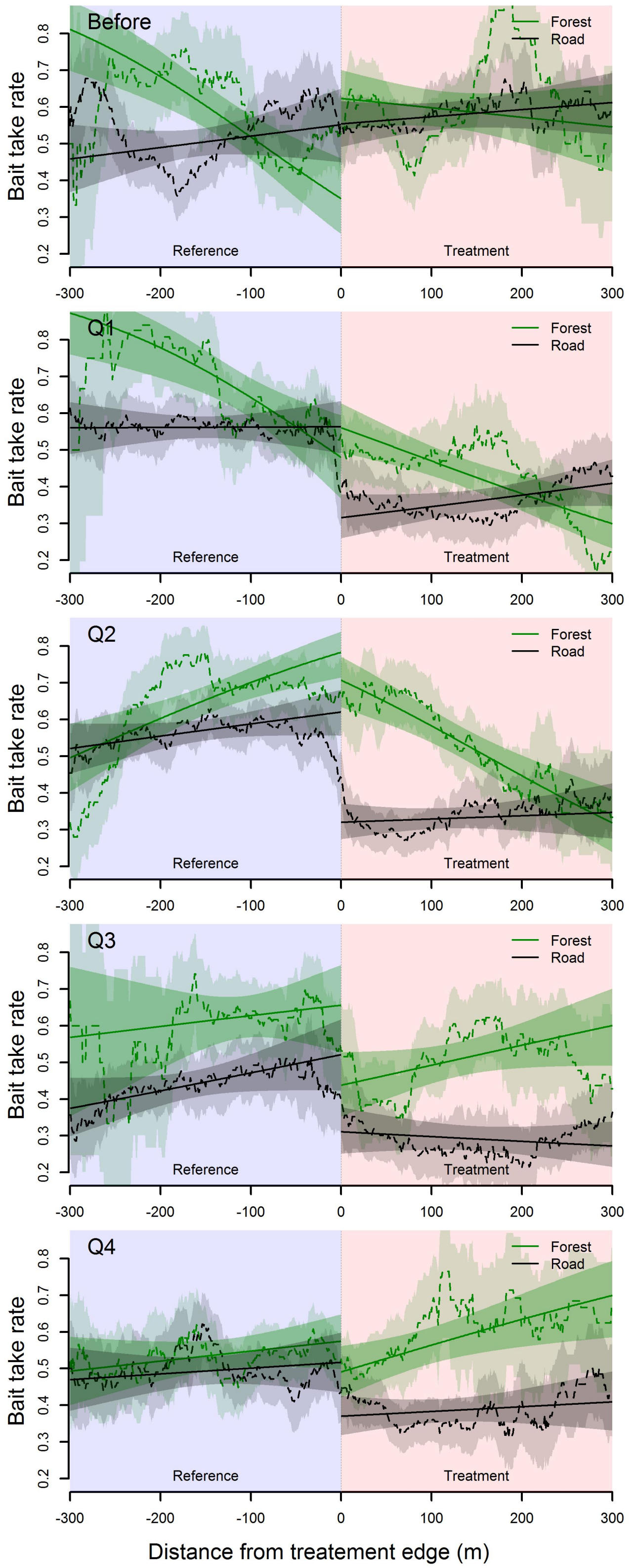

Figure 7: Spatial distributions of bait take rates across treatment borders. Dashed lines are 100-m averages, with 80% binomial confidence limits. Linear segments are slopes from a logistic regression model as predicted by distance from the treatment edge.

indicate a roughly equivalent inferred effect size.

Both of these methods, as well at the treatment itself, rely on ingestion of DNM baits. Brown Treesnake control tools that rely on a rodent lure to exploit foraging behavior have been shown to be biased in effectiveness toward larger snakes (Tyrell et al. 2007, Rodda et al. 2007, Rodda and Reed 2017). This is likely due to an ontogenetic prey shift, from feeding on small lizards as juveniles to birds and mammals as adults (Savidge 1988, Greene 1989, Mackessey et al. 2006, Lardner et al. 2009, Siers 2015). Use of DNM in bait tubes has also been shown to be less effective for smaller size classes of snakes (Lardner et al. 2013), though some small snakes do take DNM baits (C. Clark, USDA, unpublished data). Preliminary data from a current study simulating aerial bait applications in a known and geographically closed test population suggest that larger snakes are less likely to be removed in the early stages of such a suppression operation (M. Nafus, USGS, unpublished data). A shortcomings of our method for indexing relative snake abundance in our study area is that it is likely to be somewhat biased toward size classes of snakes that are prone to taking DNM baits. Additionally, unobserved bait takes give no information about the size of the snake. It is not ideal that our method for monitoring snake reduction is based on the same bait type that was used for the snake suppression treatment; an ideal study design would incorporate a completely independent metric. Further studies are currently underway to evaluate size class biases in efficacy of DNM baits, employ alternative lures to document the size classes of snakes remaining after aerial baiting operations, confirm the effectiveness of the 80-mg dose for the very largest of Brown Treesnakes, and track the survival of known snakes during aerial baiting operations using radio telemetry. We speculate that these studies will demonstrate that some size classes of Brown Treesnakes will be relatively less susceptible to aerially-delivered DNM baits as employed in this automated system. It remains to be seen whether it will simply take longer to effectively target these size classes, such as relying on aging of refractory juveniles into more susceptible adults, or whether supplemental strategies will be required, such as larger or alternative baits for very large snakes.

Management Implications

Because size distributions of Brown Treesnakes can vary by habitat (Siers et al. 2017b) and in their relative invasion risk (Siers et al. 2017c), and survival of very large snakes would likely impede successful reintroductions of native birds, it will be of fundamental importance to continue to evaluate the demographic effects of sustained aerial treatments on Brown Treesnake demographics. At this time, USDA Wildlife Services, the service provider implementing this tool, makes no claims that ADS alone will be an effective tool for eradication.

Although Brown Treesnakes can and do cross roads, they do so less than would be expected if roads were truly neutral landscape features (Siers et al. 2014). Roads are a major feature of habitat fragmentation, and often form administrative boundaries. The apparent short-term effect of roads slowing reinvasion from surrounding habitats indicated in our study will be helpful in maintaining suppression in treatment areas that are bounded by roads. The roads demarcating our treatment area on three sides were relatively minor, being narrow, without broad shoulders, and with very low traffic levels in a little-used, restricted-access military facility. The rates at which snakes cross roads have been shown to decrease with increasing road "magnitude," characterized by increasing road surface width, habitat gap width, and traffic volume (Siers et al. 2016). Major roads are likely to pose greater impediment to reinvasion and be useful boundaries for landscape-scale snake suppression treatment units.

Pulses in rat and shrew abundance have been observed where snake numbers have been experimentally suppressed (USDA and USGS, unpublished data). No increase in rat captures was associated with this experimental suppression of snake abundance. The suppressive effect of this single event was relatively modest, and it is likely that rodent population recovery would result from more intense and sustained Brown Treesnake suppression. Because of the potential negative consequences of "mesopredator release" (Crooks and Soule 1999) on invasive rat populations following removal of predation pressure from Brown Treesnakes, this should be considered a potential risk to natural resources, human health, and prospects for further success of Brown Treesnake control measures.

The results of Siers et al. (2017a, in press) and the study reported here have been interpreted as a successful proof of concept for automated bait production and aerial delivery for landscape-scale reduction of Brown Treesnake abundance. With subsequent engineering improvements, fabrication of production-grade manufacturing components, and augmented capacity through production of an additional ADS unit, USDA Wildlife Services now has a new technology for Brown Treesnake interdiction and damage mitigation in their toolbox. The first operational bait applications for sustained Brown Treesnake suppression are currently scheduled to occur in late 2018 within a 55-ha snake exclosure on Andersen Air Force Base. The objectives of this Habitat Management Unit (HMU) include using the site as a testing ground to evaluate the potential for small-scale Brown Treesnake eradications and for native species restoration (Siers and Savidge 2017). These promising results have catalyzed the formation of an informal multi-agency Guam Bird Restoration Group to identify additional research needs and to evaluate the potential for experimental reintroductions of native birds previously extirpated by Brown Treesnake predation.

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