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Efficacy of aerial broadcast baiting in reducing brown treesnake numbers

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***Abstract*:** The brown treesnake (*Boiga irregularis*) is an invasive predator that was introduced on Guam as a stowaway in cargo after World War II. Since then, the population has exploded, attaining 50 to 100 snakes per ha in some areas. The snake has caused the extirpation of ten of the 12 native forest bird species on Guam. The U.S. Department of Agriculture, Wildlife Services, has a program to deter the spread of snakes from Guam to other islands. Hand capture from fences, trapping, toxic bait stations, and canine inspection of outbound cargo methods are used in the control program in various localized and accessible areas. We investigated aerial delivery of toxic baits as a potential method for a broader landscape control of brown treesnakes. Treated baits were deployed on 6-ha of forest at 37.5 baits per ha. Snake activity was reduced by 80 to 85% by the third application of toxicant. Nontarget bait-take was limited. Of 80 telemetered baits aerially deployed, 30 (38%) baits were taken by snakes, one was taken by a toad (*Bufo marinus*), and one was taken by a monitor lizard (*Varanus indicus*). Mortality was observed in all 30 cases of bait-take by the snakes. No evidence of ill effects was observed in the toad or the monitor lizard after bait ingestion. Aerial delivery of toxic baits holds promise as an economical, targeted method to control invasive brown treesnakes over large areas of land.

***Key words*:** acetaminophen, aerial delivery, *Boiga irregularis,* brown treesnake, Guam, human–wildlife conflicts, invasive species, snake, toxicant

Brown treesnakes (*Boiga irregularis*) are invasive to the island of Guam and are believed to have arrived between the late 1940s or early 1950s via cargo transport (Rodda et al. 1992, Fritts 1988). Since that time, they have caused the decline and extinction of native birds and reptiles (Savidge 1987, Rodda and Fritts 1992). Because of the ecological and economic risk and impact this invasive species poses to other Pacific islands (Fritts 1987, 1988, Kaiser and Burnett 2010, Shwiff et al. 2010), the U.S. Department of Agriculture, Wildlife Services (WS), has put in place a program of containment and localized control around ports, airports, and military bases where the risk of snakes being transported off the island is highest (Vice and Pitzler 2002, Colvin et al. 2005).

The WS program employs a variety of methods to aid in the control of brown treesnakes. Static and mobile barriers have been employed to protect vulnerable natural resources and as temporary quarantine structures for mobile transport units (Perry et al. 1998, Campbell 1999, Aguon et al. 2002). Detector dogs are used for cargo inspection (Engeman et al. 1998*a*, 1998*b*; Vice and Vice 2004; Vice et al. 2009). Chemical fumigants and thermal treatments have been developed for killing snakes hiding in cargo (Brooks et al. 1998*a*, Savarie and Bruggers 1999, Savarie et al. 2005, Perry and Vice 2007). Nonlethal fumigation using repellents has been used to force snakes out of hiding places (Clark and Shivik 2002). Dermal and oral toxicants have been evaluated in the lab and field. Spotlight search-and-capture along fences at strategic locations has been used as an economical method for removal of snakes from areas (Engeman et al. 1998*c*, Engeman and Vice 2001). Traps with live-mouse lures account for most snake control efforts and are used to capture snakes along forest edge, in and around buildings, and along fence lines (Linnell et al. 1998, Vice et al. 2005).

Long-term trapping of moderately sized habitat plots (~17 ha) can reduce snake populations significantly, but this is a logistically intense and costly effort that can be used practically only along habitat perimeters (Engeman et al. 2000). Costs and labor allocation are always of concern in any operational program, but especially so when large areas or rugged, inaccessible terrain needs to be managed. A lower cost alternative to trapping has been proposed that uses dead mice baits treated with acetaminophen (Savarie et al. 2001, Clark et al. 2012). Acetaminophen currently is used operationally in bait stations, but stations are limited to areas of the forest adjacent to trails and roads that are easily accessible. To further improve control efficiency and reduce costs, mass aerial delivery of toxic baits over large landscapes has been proposed (Shivik et al. 2002). Studies for control of rodents on islands has shown the cost effectiveness of aerial control efforts (Howard et al. 2007). Our study reports on improving operational efficacy of aerially delivered toxic baits as a critical next-step link toward the goal of developing a method for large area control of brown treesnakes. Specifically this study evaluated the efficacy of aerial delivery of acetaminophen-adulterated mouse baits (AMB) at reducing brown treesnake populations and determined the nontarget risks of AMBs when these baits are deployed without the protection of bait stations.

Study area

The study was conducted on Northwest Field, Andersen Air Force Base, Guam (13°37′N, 144°51′E). Primarily used during World War II, the runway area has not been continuously active since 1949. Following World War II the area has been used for a variety of military training exercises. The study area is described as limestone forest and is the largest expanse of such habitat on Guam (Perry and Morton 1999). The study plots have been characterized as secondary-forest consisting of *Morinda citrifolia, Hibiscus* spp, *Premna obtusifolia, Pandanus fragrans, Aglaia mariannensis, Leucaena leucocephala.* A detailed description of the flora and fauna of the study area can be found in Perry and Morton (1999).

Methods

Toxicant

After screening numerous candidate toxicants (Brooks et al. 1998*b*, 1998*c*; Savarie and Bruggers 1999), we determined acetaminophen to be the toxicant of choice based upon criteria of efficacy (Savarie et al. 2000) and environmental safety (Johnson et al. 2002). Acetaminophen is registered (U.S. EPA Reg. No. 56228-34) as an oral toxicant for operational use (Savarie et al. 2001, Johnston et al. 2002). Toxicant tablets were formulated at the National Wildlife Research Center laboratories as follows: 73% by weight-active ingredient, acetaminophen (CAS# 103-90-2, Aerchem Inc., Lot No.: 4ACP0803-1) combined with inactive ingredients and pelletized into tablets. Inactive ingredients in the toxicant tabletswere: 3% polyvinylpyrrolidone, cross-linked (CAS# 25249-54-1), 1% carboxymethyl cellulose, sodium salt (CAS #9004-32-4), 18% microcrystalline cellulose (Avicel®) (CAS# 9004-34-6); 4% calcium phosphate, dibasic anhydrous (CAS #7757-93-9), 0.3% magnesium stearate (CAS 557-04-0), and 0.6% stearic acid (CAS #57-11-4). The ingredients were formulated into 40-mg active ingredient tablets. Chemical assay (mean ± s.d.) of 7 tablets was 41.8 ± 1.0 mg acetaminophen.

Bait

Brown treesnakes in the wild readily accept dead mice as food across all locations and seasons (Shivik and Clark 1997, 1999), and AMBs can be used to reduce snake numbers in small forested areas when placed in bait tubes (Savarie et al. 2001, Clark et al. 2012). However, an aerial drop of toxic baits posed several challenges, among which were adequate access and dosage for target animals. An 80-mg dose is 100% fatal to brown treesnakes within 3 days (Johnson et al. 2002). Two 40-mg acetaminophen tablets were inserted into the throat of dead neonatal mice (Essex Exotics and Pets, Blum, Tex.) to achieve a lethal dose for brown treesnakes from a single ingestion of bait. A previous study using mouse baits fitted with radio transmitters determined that brown treesnakes took an average of 1.1 untreated baits after aerial delivery (Shivik et al. 2002).

Bait delivery

Although brown treesnakes can be found at any strata in an ecosystem, they are predominately arboreal. To increase the likelihood that baits would get caught up in the canopy, AMBs were glued onto corn starch streamers and frozen at -15° C until ready for field deployment. The entire delivery system was designed to entangle in vegetation during aerial delivery and biodegrade within 2 to 4 days in the event that a snake did not consume the bait (Savarie et al. 2007).

The study was conducted under a Quarantine Exemption issued by the U.S. Environmental Protection Agency under Section 18 of the

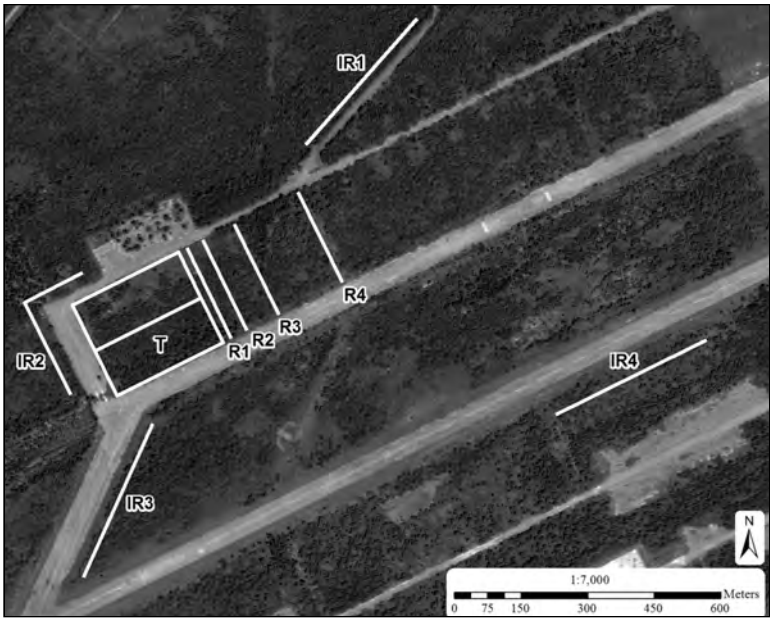

Figure 1. Aerial image of the study area on Northwest Field.

Federal Insecticide, Fungicide and Rodenticide Act. The study was designed to incorporate temporal replication and comparison to reference sites to look for potential treatment carryover effects along spatial gradients. The study was comprised of a 6-ha treatment plot where the AMBs were deployed for snake control. Adjacent to the treatment area, and within the contiguous vegetation block, 4 reference transects (R1 to R4) were established with bait-monitoring stations at distances of 20, 60, 140, and 300 m from the edge of the treatment area (Figure 1). These reference transects were designed to detect spatial carryover effects of the treatment within the contiguous patch of vegetation. In addition, 4 isolated reference transects were established in isolated vegetative blocks (IR1 to IR4). These isolated reference transects were designed to look at treatment block carryover effects in habitat separated by roads and runways that snakes reportedly are reluctant to cross.

The aerial application of AMBs was achieved by having a Navy CH-46 Sea Knight helicopter fly above the treatment plot at a sufficient height to minimize rotor down-wash effects and to allow AMBs with streamers to unfurl and fall into the vegetation. Baits were manually deployed from the helicopter which flew transects over the treatment plot. Four aerial drops of AMBs occurred on cumulative test days (CTD) 8, 16, 24, and 32 (Figure 2). A total of 225 AMBs was applied to the treatment area (6-ha) for each aerial bait drop. The application rate of AMBs was equivalent to 37.5 baits/ha. We estimated that this application rate would be sufficient to substantially impact the brown treesnake population on this plot, given assumptions of snake density of 50 to 100 snakes/ha (Rodda et al. 1999) and an ingestion rate of 1.05 baits/snake (Shivik et al 2002). On CTD 40, 42, and 44, AMBs were placed in bait tubes on the treatment plot to eliminate snakes that entrained (i.e., became conditioned) to PVC tubes, and 20 radio-telemetered AMBs were hung in trees on the treatment plot to simulate an aerial drop on CTD 46 and ascertain the effectiveness of the baiting program (Figure 2). We estimated that a total of 300 to 500 snakes would be killed.

Indices of snake activity

Two indices were used to assess efficacy of the toxicant at reducing snake numbers. The first measure of efficacy consisted of placing radio transmitters (Model F1620, Advanced Telemetry Systems, Isanti, Minn.) in AMBs (*n* = 20/drop, or 10% of the total deployment), and tracking the fate of those baits in the days following the bait drop. Immediately after the AMB drop, and at 24-and 48-hour intervals, the baits were geo-located, and their positions were marked with flagging. This activity allowed an assessment of the rate of bait acceptance by targets (brown treesnakes) and nontargets (other animals), movement of animals that ingested bait, and their fate. All carcasses and unconsumed baits with transmitters were recovered after 48 hours.

The second index of snake activity consisted of monitoring the disappearance of unadulterated mouse baits (UMBs) placed in 30-cm sections

Figure 2. Temporal schematic of the study. CTD = cumulative test day; T = treatment; R = reference sites; IR = isolated reference sites; P= unadulterated mouse bait; AD = aerial drop of adulterated mouse bait; SAD = simulated aerial drop (hand placement of bait).

CTD	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52
T	p	p	p	AD	p	p	p	AD	p	p	p	AD	p	p	p	AD	p	p	p	b	b	b	SAD	p	p	p
R	p	p	p		p	p	p		p	p	p		p	p	p		p	p	p	p	p	p	p	p	p	p
IR	p	p	p		p	p	p		p	p	p		p	p	p		p	p	p	p	p	p	p	p	p	p

of 10-cm diameter PVC tubes along transects (Figure 1). Based on 1,000 hours of video analysis, acceptance of bait from PVC tubes was almost exclusively due to brown treesnakes (L. Clark, U.S. Department of Agriculture, personal observation). The tubes were hung about 1.5 m high in vegetation and placed at 20-m intervals, with 62 tubes on the treatment area transect, 12 tubes per transect on transects R1 to R4, and 20 tubes per transect on transects IR1 to IR4. UMBs were placed in all tubes at the beginning of the study (day 0). Every 2 days thereafter, all PVC tubes were checked for presence or absence of UMBs (Figure 2). At that time, new UMBs were placed in all tubes, and old UMBs were removed. The exception to this schedule occurred during the AMB drop when no UMBs were placed in any PVC tubes (Figure 2). This was done to preclude interference in access and uptake of the AMB.

Comparisons between the UMB take indices were made between the treatment area and reference transects at 20, 60, 140, and 300 m using a 1-sided binomial comparison of proportions averaged over the time period CTD 42 to 52. Similar comparisons were made between the bait take of AMBs and the bait-take from UMBs in the treatment plot (Hill and Lewicki 2007).

Results

Impact of aerial delivery of AMBs on snake activity within the treated plot

Of the 80 telemetered AMBs aerially delivered, thirty (38%) were taken by snakes, 1 was taken by a cane toad, and one was taken by a monitor lizard. Neither the toad nor the monitor lizard showed any evidence of ill effects from ingesting the bait. All of the 30 snakes that consumed the bait-telemetered AMB died. Snake carcasses were recorded within 10 to 20 m of the original location of the AMB aerial drop. All mortality occurred within 48 hours of AMB consumption. There were no multiple consumptions of telemetered AMBs by the same snake. The take rate of telemetered AMBs decreased after each drop, suggesting that the overall toxicant drops were negatively impacting the number of snakes available to take telemetered baits (Figure 3). Of the 20 radio-telemetered baits hand placed in trees at the end of the study, three were taken by snakes.

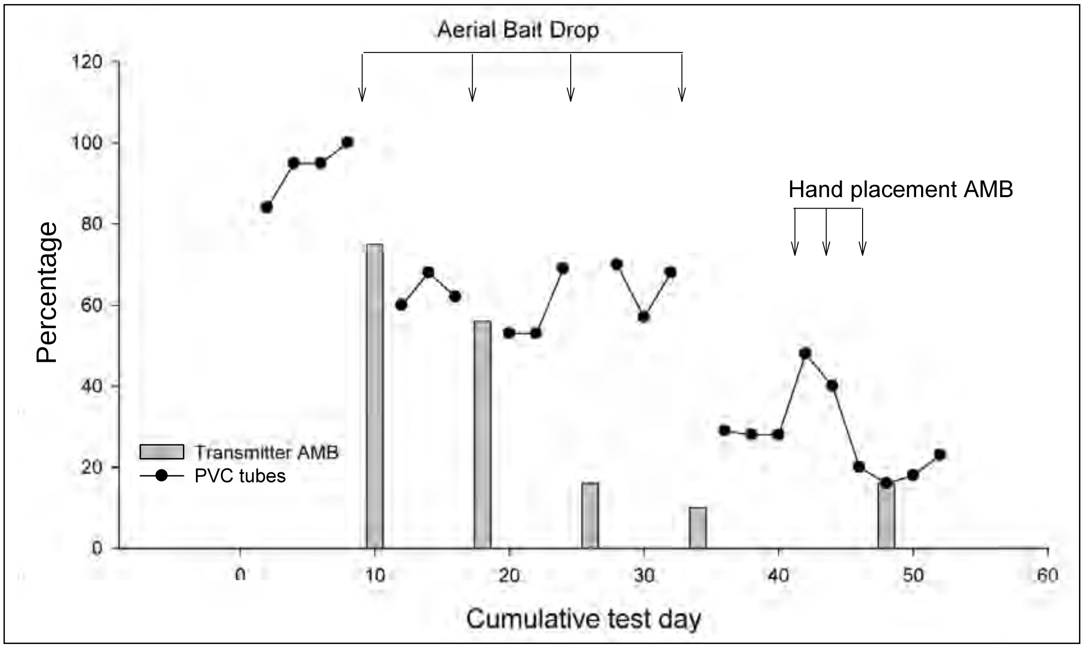

Figure 3. The percentage of unadulterated mouse baits (UMB) taken from PVC monitoring tubes on the treatment plot (symbols) and the percentage of telemetered acetaminophen adulterated mouse baits (AMB) taken after each aerial drop on the treatment plot and the simulated aerial drop (SAD).

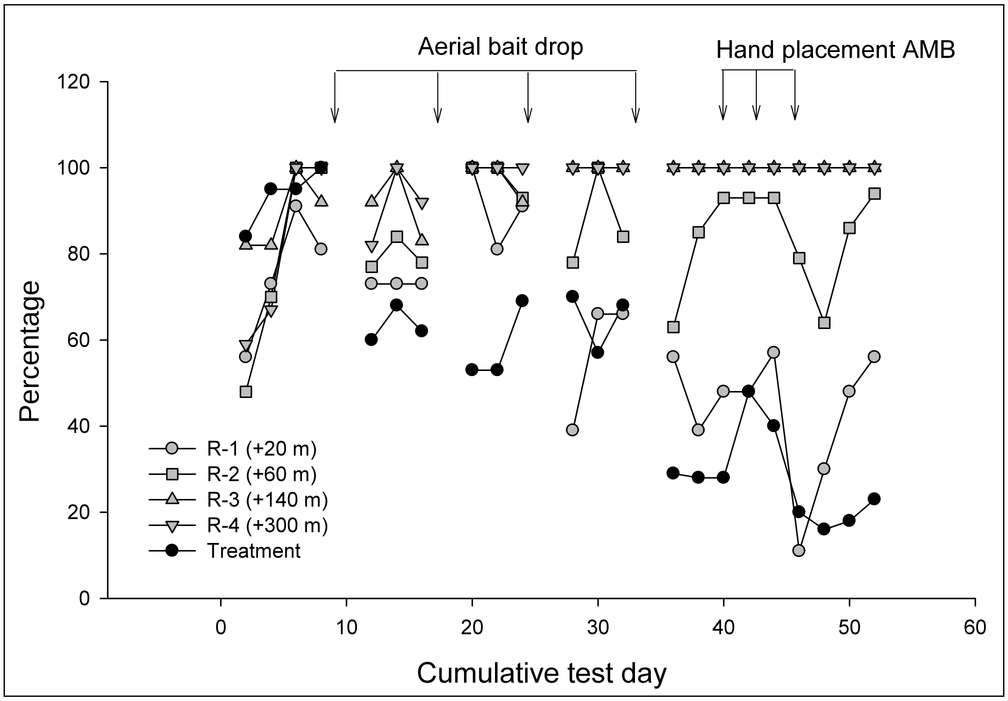

Figure 4. The percentage of baits taken from monitoring tubes positioned on the reference plots and the treatment plot.

Prior to the aerial toxicant delivery, 98% of UMBs were taken from PVC tubes on the treatment plot. After the first 3 AMB drops, approximately 60% of UMBs were taken from PVC tubes (Figure 3). After the fourth AMB drop, 28% of UMBs were removed from PVC

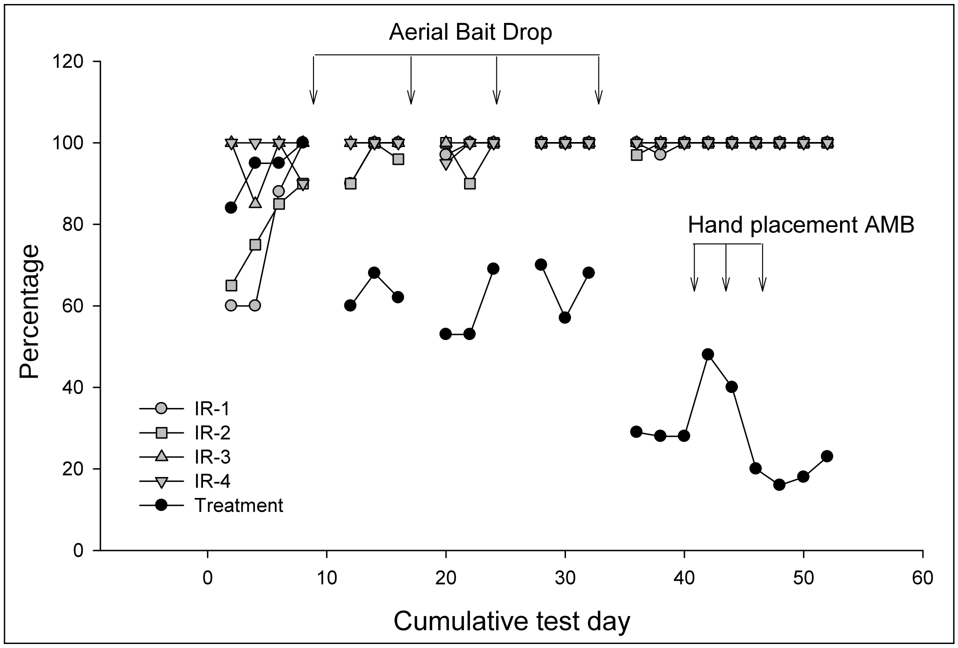

Figure 5. Percentage of baits taken from monitoring tubes positioned along the periphery of isolated reference plots and the treatment plot.

tubes. These observations suggest that aerial delivery of toxic baits did impact take of UMBs from PVC tubes. However, the take rate from PVC tubes was higher than indicated by the take rate of telemetered AMBs (Figure 3). This observation suggested the possibility that some snakes may have entrained to the tubes as a source of food and restricted their movements, thus decreasing their likelihood of encountering an aerially dropped AMB. Hand-placement of AMBs in PVC tubes at the end of the aerial application period (CTD 40 to 44) supports this interpretation. After hand-placement of AMBs in PVC tubes the take rate of UMBs from those tubes on subsequent days and the simulated aerial drop (CTD 48 to 52; Figure 3) was 15%. This was more in line with the bait-take rate for aerially dropped, telemetered AMBs (z = 2.24, P = 0.81).

Impact of aerial delivery of AMBs on snake activity on adjacent areas within the treated plot

The aerial drop of AMB's affected snake activity in close-by, nontreated, adjacent vegetated areas (Figure 4). The average take-rate of UMBs for the aerial treatment plot and the 20-m reference transect were similar during CTD 42 to 52 (z = -1.12, p = 0.13), suggesting treatment was affecting nearby snake activity in untreated areas. At 60 m from the treatment plot, the take rate for UMB was slightly higher in the reference transect (z = 1.97, P = 0.03), suggesting that the treated area was having a diminished effect on more distantly located snake activity. At 140 and 300 m from the edge of the treated plot, the index of snake activity was substantially higher than the treatment plot (z = 2.19, P = 0.02), suggesting that snake activity at this distance was not negatively affected by aerial deployment of AMBs. Combined, these results suggest that within the time frame of the study, snakes have a limited range of movement, and that the inferred lethal effects on the treatment plot were not impacting snakes at distances >60 m. The consequence of this limited movement is that AMB deployment will affect snakes only within a small radius. This radius appears to be 0 to 60 m.

Impact of aerial delivery of AMBs on snake activity in isolated plots

There was no apparent effect of the aerial drop of AMBs on snake activity in isolated reference plots (Figure 5). The take rate of UMBs for IR1-IR4 was near 100%. This compared to a UMB take rate that decreased with time in the AMB treated plot.

Discussion

Based on the number of toxic baits dropped onto the treatment plot and the proportion of telemetered baits taken, we estimate that 338 brown treesnakes were killed by the aerial delivery of AMBs. An additional 46 snakes were likely killed by post-drop hand placement of AMBs in bait tubes, and 3 snakes by the AMB-simulated aerial drop. We estimate that a total of 387 snakes were killed over a 52-day period on a 6-ha plot. That suggests that the plot contained at least 65 snakes/ha. The fact that there remained a low rate of bait-take at the end of the study (~15%) suggests that even more snakes were on the plot. Regardless, this minimal density estimate is within the 50 to 100 snakes/ha previously reported for Guam (Rodda et al. 1999).

The total amount of acetaminophen (88 g) placed on the landscape was the equivalent to 177 adult-strength (500 mg each) tablets of over-the-counter medicine, which is fewer than a single economy bottle of pain relief medicine (500 caplets/bottle). We previously assessed the primary and secondary hazards to target and nontarget animals resulting from acetaminophen exposure and concluded that no significant risks existed for nontarget animals as the application rates described (Johnston et al. 2002).

Bait tubes are a convenient method to present toxicant to snakes. Such tubes offer some degree of exclusion to nontarget species, protection from weather, and an easy method to document rates of bait take; but, their use is most practically restricted to perimeters of forests, buildings, and fence lines. As seen in this study, the use of bait tubes as an index of snake activity is limited. It appeared that some snakes may have learned about a static and reliable food source and restricted their movement to the exclusion of encountering randomly deployed aerially dropped AMBs. However, once those snakes were eliminated by hand baiting with AMBs, the snake-activity indices from the tubes and telemetered baits converged. We suggest that bait tubes can be reliable as both an index of snake activity and convenient method for control along perimeters if AMBs are used (Savarie et al. 2001).

Weekly pulsed application of AMBs over 4 weeks resulted in a decline in the numbers of aerially deployed, radio-telemetered AMBs consumed by snakes. This is consistent with the interpretation that there were fewer snakes on the plot available to consume baits. Thus, we conclude that aerial delivery of toxic baits is effective at reducing brown treesnake numbers in treated habitats. As in previous short-term studies, there was no evidence of eradication.

Indices of snake activity were reduced by 85% relative to pretreatment levels or as compared to adjacent and isolated reference plots. There may be several reasons why eradication was not achieved. First, for a given application rate, encounter rates by snakes may simply need time to effectively expose all snakes on the plot to toxic baits. Second, the 15% bait-take rate may reflect equilibrium between kill rates and immigration rates. We do not favor this interpretation for the time scales considered for the following reasons. Previous studies demonstrated that brown treesnakes do not travel far over short periods of time. Tobin et al. (1999) found that brown treesnakes travel between 5 to 17 miles per hour, and move from their initial site of capture, 36 to 50 m during 6 to 40 days. The patterns of movement did not differ across seasons. Shivik et al. (2002) found similar movement patterns for snakes consuming telemetered baits dropped into habitat from helicopter. The average movement of brown treesnakes from the point of ingestion was 21 m (1 to 70-m range) over a 24-hour period. Our observations on snake movement are consistent with these studies. No telemetered snake left the treatment study plot, suggesting that the reverse was also a low likelihood event. We did not detect any impact on nontreated reference transects beyond 60-m from the treated plot, again, suggesting that snakes do not move far during a limited time period. The final evidence is the lack of carryover effect of the treated plot on nearby reference transects located in adjacent habitat patches but separated by runways. Third, snakes are not active every night. Snake satiety or molt may influence movement and foraging behavior and activity (Tyrell et al. 2009). The availability of AMBs and UMBs is unlikely to profoundly influence satiety, however. Bait-take on isolated reference plots did not decrease over time, as would be expected if appetite were suppressed as a consequence of supplemental feeding with

UMBs. Moreover, all evidence indicates that snakes' exposure to AMBs resulted in their death, so satiety is a moot point. However, satiety may influence activity patterns of snakes not actively foraging at the start of a control operation. The persistent low level of bait-take at the end of control studies may reflect emergence of snakes aroused to activity when satiety abates (Savarie et al. 2001, Clark et al. 2012). The cause of this persistent low level of activity should be investigated further.

To compensate for factors responsible for variation in activity patterns, any large-scale control effort should include frequent long-term, pulsed applications of baits (i. e., 4 to 16 weeks, once per week, at an application of 36 baits per hour), followed by perimeter monitoring with AMB bait stations, as needed. Control efforts also should be geospatially integrated and systematic. As control efforts become more efficient and increasingly integrated, the risk of brown treesnakes escaping from the island will decrease. Aerial and tube delivery of ABMs ultimately will allow for lower cost programs to be put in place to reduce snake densities over large areas, allowing control programs to more efficiently deploy their intensive methods (e.g., detector dogs for cargo inspection, fence line searches, quarantine, and trapping).

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