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(RC-200925)

Aerial Application of Acetaminophen-treated Baits for Control of Brown Treesnakes

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14. ABSTRACT

The objective of the this Demonstration Project was to validate the use of aerial techniques to deploy acetaminophen–treated dead neonatal mice baits to reduce brown treesnake (*Boiga irregularis*) numbers in forested sites on Guam. A total of 29,700 baits were applied over 15 bait drops (8 on the HMU and 7 on the MSA). Canopy landing and distance between VHF marked baits was 82% and 19.3 m, respectively exceeding success metrics for both measures. The success metric of a >80% reduction in bait take rate of VHF marked baits (N=105) within 4 drops was achieved on the HMU site (83%) but not the MSA site (73%). Success metrics of maintaining bait take rates below 30% on both the HMU and MSA for at least 12 and 4 weeks respectively, were achieved. Indices of snake abundance on the HMU and MSA drop location were significantly ($P < 0.05$) less than the reference site for all bait drop periods. A total of 5 rats (*Rattus* spp.) were captured in 2,772 total trap nights of sampling on all 3 locations, indicating no compensatory increase in rodent numbers. Only two non-targets (<2%) were recovered during the 16 month field phase meeting the success metric of < 10% non-target take. Estimated cost for implementation on a 500 ha site was $5,476/ha. The demonstration indicates that this technology can be successfully used to control BTS over large areas. Further refinement of the technology such as automation of bait delivery could provide significant cost savings in operational deployment at larger scales.

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ACRONYMS AND ABBREVIATIONS

APHIS	Animal and Plant Health Inspection Service
ATOC	Aviation and Training Operations Center
BTS	Brown Treesnake
CM	Centimeter
CPUE	Catch Per Unit Effort
CFR	Code of Federal Regulations
CNMI	Commonwealth of the Northern Mariana Islands
DNM	Dead Neonatal Mouse (Mice)
DOD	Department of Defense
DOI	Department of the Interior
DOT	Department of Transportation
EPA	Environmental Protection Agency
ESA	Endangered Species Act
ESRI	Environmental Systems Research Institute
ESTCP	Environmental Security Technology Certification Program
FAA	Federal Aviation Administration
FAR	Federal Aviation Regulation
FP	Field Procedure
FTE	Full Time Equivalent
FY	Fiscal Year
GIS	Geographic Information System
GPS	Global Positioning System
H	Hour
Ha	Hectares
HMU	Habitat Management Unit
IPR	In Progress Review
Km	Kilometre
LP	Laboratory Procedure
M	Meters
Mg	Milligram
MOA	Memorandum of Agreement
MSA	Munitions Storage Area

NEPA	National Environmental Policy Act
NWRC	National Wildlife Research Center
QA	Quality Assurance
QAU	Quality Assurance Unit
REF	Reference Site
SD	Standard Deviation
SOP	Standard Operating Procedure
T&E	Threatened and Endangered
TBO	Time Between Overhaul
TIS	Time in Service
TSCA	Toxic Substances Control Act of 1976
USDA	United States Department of Agriculture
USFWS	United States Fish and Wildlife Service
VHF	Very High Frequency
WS	Wildlife Services

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EXECUTIVE SUMMARY

OBJECTIVES OF THE DEMONSTRATION

The objective of the this project was to validate the use of aerial techniques to deploy acetaminophen–treated dead neonatal mice (DNM) baits to reduce brown treesnake (*Boiga irregularis,* BTS) populations in forested sites on Guam. In particular, this project developed an operational aerial control method for depopulating snakes on a landscape level that would reduce the risk of snakes in Department of Defense (DOD) cargo facilities before they enter into areas of military transport. Aerial delivery is a technique for depopulating BTS in large forest areas. Issues validated included the number of baits that landed above ground level, number of aerial deployments for reducing treated bait take by greater than 80%, duration of bait take reductions, compensatory increases in non-native rodent abundance, and impacts to non-target animals.

TECHNOLOGY DESCRIPTION

Thawed DNM were treated by inserting an 80 Milligram (mg) acetaminophen tablet into the body cavity through the mouth. The acetaminophen tablets were a specific formulation made by the United State Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center (USDA, APHIS, WS, NWRC), chemistry formulation labs in Fort Collins, Colorado. Radio transmitters also were implanted into the body cavity for tracking a subset of the treated DNM. Treated DNM baits were individually attached to 1.2 meter-long paper flag streamers with cardboard on each end of the paper streamers, hereafter defined as flag-baits. The paper streamer was folded accordion-style between the cardboard, forming a flat, compact flag-bait. The flag-baits were packaged into trays and frozen until deployed. Flag-baits were deployed by hand from a helicopter over a prescribed forested drop zone at 36 baits per hectares (ha), the Environmental Protection Agency (EPA) registered application rate (EPA Registration #56228-34 held by WS, NWRC). The double-ended cardboard streamers form an arc in the air and entangle the treated DNM in vegetation above ground level where they are consumed by Brown Treesnakes.

Radio-telemetered DNM (a subset of the total dropped) were tracked to determine the percent landing in the canopy, distance between baits, and fate of baits. Snake activity on the Habitat Management Unit (HMU) and Munitions Storage Area (MSA) aerial drop sites (55 ha each) and one 55 ha reference site (REF) was determined by untreated DNM taken from bait stations monitored twice monthly as an index of BTS numbers. Rodent numbers were monitored quarterly through capture of rats in live traps to evaluate possible compensatory increases in rodent abundance (as BTS abundance decreases, rodent abundance may increase). Costs were tracked and scaled up to a hypothetical 500 ha drop site to estimate costs of an operational scale control program. Lastly, projected cost savings of advancements in automated bait delivery devices were estimated and applied to a 500-ha drop site operational program.

DEMONSTRATION RESULTS

A total of eight quantitative performance metrics and one qualitative performance metric for the demonstration plan were evaluated. Of the eight quantitative metrics, seven were met or exceeded, and one was marginally successful but complicated by extenuating factors. The qualitative performance metric was met.

Performance Objective Summary Table.

See subsequent narrative for details.

Performance Objective	Success Criteria	Criteria Met
Quantitative Performance Objectives		
1) Maximize landing of telemetered baits above ground level	> 80% of telemetered baits deployed above ground level across all drops. Minimum >70% of telemetered baits deployed above ground level per drop	Yes, (See narrative)
2) HMU test site: Reduce BTS abundance as measured by reduced telemetered treated DNM bait take by BTS	No more than four aerial deployments at 2 week intervals for reducing telemetered treated baits taken by BTS by >80%	Yes, (See narrative)
3) MSA test site: Reduce BTS abundance as measured by reduced telemetered treated DNM bait take by BTS	No more than five aerial deployments at 2 week intervals for reducing telemetered treated baits taken by BTS by >80%	No, (See narrative)
4) HMU test site: Reduce BTS abundance sufficiently to minimize interval between aerial maintenance deployments	At least 12 weeks between deployments for maintaining BTS bait take < 30% before next aerial drop	Yes, (See narrative)
5) MSA (no snake fence barrier aerial test site): Reduce BTS abundance sufficiently to minimize interval between aerial maintenance deployments	At least 4 weeks between deployments for maintaining BTS bait take < 30% before next aerial drop	Yes, (See narrative)
6) Minimize compensatory increases in non-native rodent abundance	< 20 % increase in rodent abundance	Yes, (See narrative)
7) Minimize non-target impacts (crabs, monitor lizards)	<10% bait take by non-target animals	Yes, (See narrative)
8) Maximize aircrew performance for delivering telemetered baits at regular intervals	Mean range of 17-23 m between telemetered baits	Yes, (See narrative)
Qualitative Performance Objective		
1) Maximize aircrew work performance during aerial bait deployment	Aircrew able to perform duties effectively with minimal boredom/fatigue	Yes, (See narrative)

Success Metric 1; maximize landing of telemetered baits above ground level. This metric was successfully met with an 82% canopy landing of baits over all sites and all drops which exceeded the greater than 80% success criteria.

Success Metrics 2 and 3; reduction in BTS abundance as measured by reduced telemetered treated DNM bait take by BTS on the HMU and MSA sites. An 83% decline in bait take rate of treated very high frequency (VHF) radio marked baits was observed on the HMU, thus reaching the success metric of an 80% decline within four drops. Reduced BTS abundance as measured by telemetered treated DNM bait take by BTS on the MSA site was marginally successful. The observed decline in bait take rate of treated VHF radio marked baits on the MSA was 73% which did not meet the success metric of an 80% decline in 5 bait drops. However, severe weather prevented bait drops on the MSA during the 3rd drop period resulting in only four bait applications during the first bait drop period. The data for the MSA site clearly indicated a decline in snake numbers and reached a 73% reduction in four applications which can be considered marginally successful.

Success Metrics 4 and 5; reducing BTS abundance sufficiently to minimize the interval between aerial maintenance deployments to 12 weeks and four weeks on the HMU and MSA sites, respectively. As was seen with telemetered baits, bait take rate from unadulterated baits in bait stations declined on both the HMU and MSA sites. The success metric of a greater than 80% reduction in bait take rate on the HMU was achieved and this average reduction was sustained for over five months. The MSA reached the target level of an 80% reduction in bait take rate and did so in four aerial bait drops and was sustained for more than four weeks. Longer-term reductions in bait take rate on the MSA were on average less than the HMU but not significantly so. In addition there was not a significant increase in bait take rate on the MSA for over seven months and the bait take rate did not exceed 30% for over eight months. Bait station monitoring indices of BTS abundance clearly show a significant decrease in snake numbers which corroborates findings from VHF marked baits. Original success metrics of maintaining bait take rates below 30% on both the HMU and MSA for 12 and four weeks, respectively were achieved. Long term bait take reductions were also achieved and drop sites were significantly less than the reference site for all drop periods. Original success metrics were met for both sites and more stringent success metrics of a greater than 80% reduction in bait take rate were met on the HMU and largely met on the MSA.

Success Metric 6; minimizing compensatory increases in non-native rodent abundance was met. A total of five rats (likely *Rattus diardii*, although species status is in question on Guam) were captured in 2,777 total trap nights of sampling on all three locations. Given that rodents were rare and their numbers did not increase, rodent live-trapping results were not statistically analyzed.

Success Metric 7; minimizing non-target impacts (crabs, monitor lizards) was met. Only one non-target, an invasive cane toad (*Rhinella marina*), was found to have consumed a VHF marked toxicant bait of the 105 deployed and recovered. An additional non-target, a juvenile monitor lizard (*Varanus indicus*), was discovered opportunistically, tested, and found to have been exposed to acetaminophen baits. Counting both of these species the success metric of a less than 10% non-target bait take rate was successfully met.

Success Metric 8; maximizing aircrew performance for delivering telemetered baits at regular intervals, was met. The bait delivery success metric of a mean distance between baits of 20 meters (m) was successfully met with an actual average distance between baits of 19.3 m (N=105).

Qualitative Performance Objective 1; maximize aircrew work performance during aerial bait deployment was met. The aircrew's success was demonstrated by the successful completion of demonstration project activities. Crews maintained production levels and enthusiasm for all aspects of the demonstration project through all project phases including: (1) study site establishment of 162, 210 m transects (34 Kilometres (km) total), placement of 1,782 bait stations on transects, (2) preparation of 29,700 flagger baits, (3) radio tracking and recovery of 105 VHF marked baits, (4) 15 total aerial bait drops of 1,980 baits each (29,700 total), (5) twice monthly baiting and monitoring of 198 randomly selected bait stations over 24 sessions (6,723 bait stations total), (6) 2,777 total trap nights of rodent sampling on all three locations, and (7) transect and snake barrier maintenance. These activities were among their core duties and do not account for data entry, training, safety, preparation, maintenance, supply acquisition, and the many other ancillary duties involved with the project. All this was done by a core technical staff of five people and one field supervisor. The superlatives to describe the successful and safe conduct of the demonstration project by the core crew cannot be exaggerated and are a testimony to the core crew's performance.

IMPLEMENTATION ISSUES

A substantial regulatory burden must be addressed prior to any field operation. All field operations to control Brown Treesnakes would be subject to the federally required National Environmental Policy Act (NEPA) process. Wildlife Services requirements are under APHIS NEPA Implementing Procedures at 7 Code of Federal Regulations (CFR) Part 372.5(c)(2)(i). This CFR encompasses projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect Threatened and Endangered (T&E) species, and projects with uncertain environmental impacts. The NEPA document would need to be reviewed and supported by the Department of Interior U.S. Fish and Wildlife Service (DOI, USFWS), including a Biological Opinion and Section 7 Endangered Species Act consultation. A federal take permit would need to be obtained from the DOI-USFWS and a permit from the Guam Department of Agriculture for application of toxicant baits. In addition any staff involved in toxicant application will need to take and pass the Guam Department of Agriculture, Pesticide Applicators Examination. Because of WS's unique position dealing with wildlife damage issues, they have their own NEPA staff to address NEPA requirements for large scale control actions.

Currently flag-baits are deployed by hand from a helicopter at 36 baits per ha, the EPA registered application rate (Registration #56228-34) currently held by the WS, NWRC. Any changes to the application rate would need approval through the EPA which can take 2-3 years. The WS, NWRC technology transfer program can aid in this process to obtain approval for changes, potentially reducing the timeframe to approval.

All aerial operations (contractual or in-house) involving WS staff must have a pre-operational safety review of pilot and aircrew per WS Aviation Training and Operations Center (ATOC). All aircraft must be maintained in accordance with Federal Aviation Regulation (FAR) part 135 and FAR Part 91 as applicable, to include 100 hour/annual inspections and compliance with the manufacturer's recommendations for Time Between Overhaul (TBO) and Time In Service (TIS). All aircraft and operators covered by this agreement must be certified under the provisions of FAR Part 135, "Operating Requirements: Commuter and On-Demand Operations and Rules Governing Persons on Board Such Aircraft." In addition to Federal Aviation Administration (FAA) requirements, all aircraft involving WS staff must conform to requirements of the WS-ATOC safety manual and program.

There was a single private contract supplier for helicopter services with capability for doing bait drops on Guam. Costs associated with this service were much greater than anticipated (See Section 7 Cost Assessment). Meeting the demands of larger scale programs and sole source contractual costs may be problematic. Wildlife Services operates their own fixed and rotary wing aircraft and as such have fully trained support staff, pilots, aircrew and policy and procedures to support flight operations. If large scale operations justifying use of WS aircraft are implemented this could facilitate field performance of operational programs.

Other regulatory considerations include Executive Order 13112 (February 3, 1999) – Invasive Species; Brown Tree Snake Control and Eradication Act of 2004 – H.R. 3479, Section 4; Defense Transportation Regulation Part V Chapter 505 Agricultural Cleaning and Inspection Requirements (29 September 2006); Brown Tree Snake Control and Interdiction Plan, Prepared by: Commander U.S. Naval Forces Marianas, Facilities and Environment N45, August 2004; and Andersen Air Force Base 36 Wing Instruction 32-7004 – Brown Treesnake Management, 15 March 2006.

These regulations and directives are for the control of BTS but do not specifically call for the development of aerial delivery of treated baits for landscape control of snakes. Modification may be necessary to encompass large scale operational control programs on Guam.

Other non-regulatory implementation issues include cost of deployment of toxicant baits and monitoring associated with a large scale operational program. Wildlife Services and NWRC and public and private partners are currently working on technologies to help develop refinements to the technology that could result in significant cost savings such as automated technologies. These ideas are discussed in Section 7 Cost Assessment.

Non-target bait take would be a concern in any application of toxicant baits to control BTSs. Addressing the application and monitoring issues above and having an effective monitoring program would be necessary for any operational program. The Demonstration Project indicated that at least on relatively isolated DOD lands, non-target bait take rate was very low. However, expansion of programs into different areas and habitats could increase non-target risk. These risks would need to be evaluated as part of the NEPA and permitting process and may require further research to evaluate. Addressing these issues proactively would reduce potential for delay in initiating operational programs.

Scaling up operational programs may entail toxicant and bait procurement issues. Currently the NWRC is the only manufacturer of the acetaminophen tablets for use in aerial bait drops. NWRC could easily meet demand for tablets on the aerial bait drop but larger scale operations may require expansion of staff, facilities and equipment to meet demand. While equipment for hand baiting was readily available, equipment being developed for automated delivery of baits is not. All automated equipment is custom built and unique to this application and would likely need to be sole sourced to the supplier. This sole supplier situation is likely to remain the case in this niche market without substantial commercial growth potential. Currently automated delivery and equipment are in a research test phase of development by WS, NWRC.

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1.0 INTRODUCTION

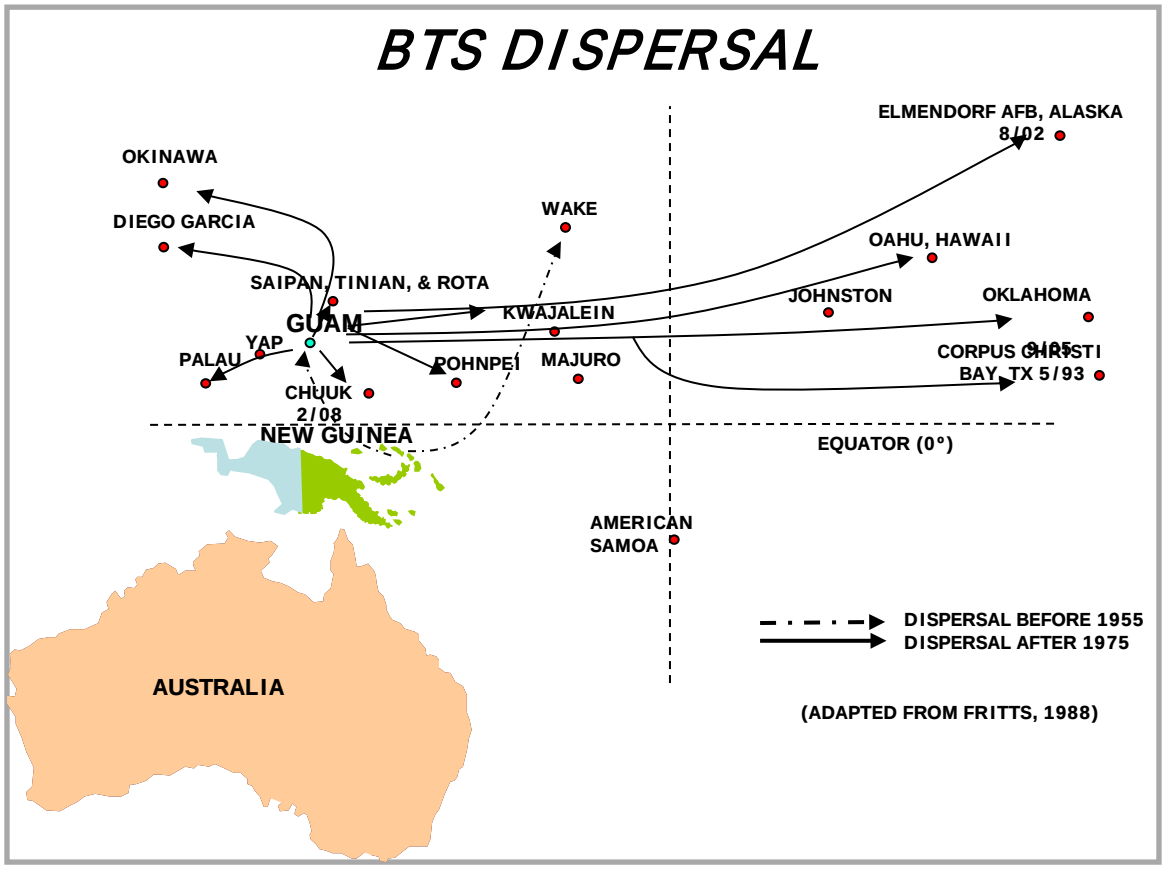
The goal of this demonstration project was to evaluate the aerial deployment of toxic baits in forests as a means to reduce Brown Treesnake (*Boiga irregularis*, BTS) numbers. Brown Treesnakes were inadvertently introduced to the island of Guam after World War II as passive stowaways in cargo and have caused a major ecological disaster. Snakes have extirpated all but two of the 12 native forest birds and have caused electrical power outages that disrupt public, commercial, and military activities. Guam is the focal point of military and commercial air and ship cargo traffic in the tropical western Pacific and there is the threat that snakes could be inadvertently introduced and establish breeding populations on other snake-free islands in the region. Sightings or captures of Brown Treesnakes of probable Guam origin include Oahu in Hawaii, and Rota, Tinian, and Saipan of the Commonwealth of the Northern Mariana Islands (CNMI). The dramatic expansion of the U.S. military presence on Guam will increase the flow of outbound cargo, increasing the risk of snake dispersal. This expansion could overtax the present operational control methods (trapping, hand capture of snakes, toxic bait stations, and canine inspection of outbound cargo) for deterring the spread of snakes from Guam. Except for canine inspection, the current control methods reduce snake populations in accessible forest and intercept snakes from the forests adjacent to cargo areas, but do not appreciably depopulate snakes in inaccessible forests. This demonstration utilized thawed dead neonatal mouse (DNM) baits treated with the BTS oral toxicant, acetaminophen, attached to paper flag streamers (U.S. Environmental Protection Agency [EPA] Registration Number 56228-34). The baits were deployed by hand at 36 baits per hectare (ha, 1 ha = 2.47 acres) from a helicopter over two 55 ha aerial test sites. The baits entangle in the canopy forest vegetation where they can be consumed by snakes. The aerial baiting technology will provide the U.S. Department of Defense (DOD) two major benefits for reducing environmental and economic risks caused by BTS. First, it will provide an additional substantial level of defense against snakes getting into outbound military cargo by reducing snake populations adjacent to cargo facilities. Secondly, this technology is unlimited in an operational sense. While currently only demonstrated on DOD properties, there is the potential for aerial application to all areas on Guam. BTS operational control has been ongoing since 1993 and will extend into perpetuity unless island-wide control is initiated because the current control methods (trapping, hand capture, bait stations, and canine detection) are not appropriate for all terrain types and at very large landscape scales.

1.1 BACKGROUND

The BTS is a nocturnal, arboreal invasive predator on the island of Guam that was probably introduced after World War II as a passive stowaway in cargo from the Admiralty Islands north of New Guinea (Fritts 1988, Rodda et al. 1992). Lacking natural predators on Guam, the population of BTS exploded, reaching as many as 50-100 snakes per ha in some areas (Rodda et al. 1992). Snakes colonized the entire island of Guam (54,930 ha) in about 20-30 years (Savidge 1987). The brown treesnake has gained the unfortunate distinction of being the only reptile known to have caused the extinction of another vertebrate species. Ten of 12 native birds have disappeared from the forests on Guam and the impact of the snake has also been devastating to the island's lizards (Rhodda and Fritts 1992). In addition, this cryptic, mildly venomous snake has caused millions of dollars in damages to the island's electrical infrastructure, bitten hundreds of people, and is a health threat to infants and young children (Fritts 1988, Fritts 1990, Fritts and McCoid 1999).

The abundance of snakes on Guam, coupled with their tendency to seek daytime refuge in cargo, creates significant risk of dispersal from the island (Vice and Vice 2004, Figure 1). Guam is the focal point of air and ship cargo traffic in the tropical western Pacific and there is the threat that snakes could be inadvertently introduced and establish breeding populations on other snake-free islands in the region. Although no incipient populations are known, sightings or captures of BTSs associated with commercial and military cargo traffic from Guam have been recorded on Oahu in the Hawaiian Islands, Kwajalein in the Marshall Islands, Pohnpei and Chuuk in the Federated States of Micronesia, Diego Garcia in the Indian Ocean, Okinawa in the Ryukyu Islands of Japan, Rota, Tinian, and Saipan of the CNMI, Texas, Oklahoma, and Alaska in the U.S. and as far away as Spain (Fritts et al. 1999). The continued movement of BTS from Guam threatens the biodiversity and economic security of the region (Savidge 1987, Vice and Vice 2004). If BTS became established in Hawaii, the potential economic damage is estimated to be $593 million to $2.14 billion per year (Shwiff et al. 2010).

Figure 1. Dispersal of Brown Treesnakes from Guam through the Transportation System



In 1993, the U.S. Department of Agriculture (USDA), Wildlife Services (WS), initiated an operational program to deter the spread of snakes from Guam using hand capture from fences during nighttime spotlight searches, trapping, and inspection of cargo with search dogs (Hall, 1996). Ongoing control efforts in and around Guam's outbound cargo areas continue to capture thousands of snakes annually, and the problem remains critical on all Guam's military installations – in Fiscal Year (FY)2009, 2,736 BTSs were intercepted on Navy installations, and 7,555 on Air

Force installations (D. Vice, pers. comm.). The majority of these were intercepted around the Navy wharves and cargo staging sites around Apra Harbor, and around the flight line, warehouses, and cargo staging facilities at Andersen Air Force Base. Adjacent to the cargo areas are forests that are inaccessible for operational control using traps and bait stations. These forests are the likely source of snakes that could disperse off-island through outbound cargo. With the pending increase of U.S. military presence on Guam, there will be increased movement of personnel and household goods, which will increase the potential for dispersal of BTS through outbound cargo. Of particular concern is the deployment of military personnel and equipment from Guam to the island of Tinian in the CNMI for training exercises and increased traffic through Hawai`i. Aerial application of treated baits in these inaccessible forests will reduce snake populations in large areas adjacent to commercial and military warehouses and cargo staging facilities, and this will reduce the risk of snake dispersal to at-risk locations such as Tinian and Hawai`i.

1.2 OBJECTIVE OF THE DEMONSTRATION

The objective was to demonstrate the use of aerial techniques to deploy acetaminophen–treated DNM baits to reduce snake populations in forested sites on Guam. The overall objective was to develop an operational aerial control method for depopulating snakes on a landscape level which would reduce the risk of snakes in DOD cargo facilities before they enter into areas of military transport. Aerial delivery is a technique to depopulate snakes in large forest areas. Issues that were validated were number of baits that land above ground level, number of aerial deployments for reducing treated bait take by ≥80%, compensatory increases in non-native rodent abundance, and impacts to non-target animals.

1.3 REGULATORY DRIVERS

Memorandum of Agreement (MOA) on Brown Treesnake Control (1999, signed by Department of the Interior [DOI], DOD, USDA, Department of Transportation [DOT], and Governors of Guam, CNMI, and Hawaii; new MOA was developed); Executive Order 13112 (February 3, 1999) – Invasive Species; Brown Tree Snake Control and Eradication Act of 2004 – H.R. 3479, Section 4; Defense Transportation Regulation Part V Chapter 505 Agricultural Cleaning and Inspection Requirements (29 September 2006); Brown Tree Snake Control and Interdiction Plan, Prepared by: Commander U.S. Naval Forces Marianas, Facilities and Environment N45, August 2004; and Andersen Air Force Base 36 Wing Instruction 32-7004 – Brown Treesnake Management, 15 March 2006. These regulations and directives are for the control of BTS but do not specifically call for the development of aerial delivery of treated baits for landscape control of snakes.

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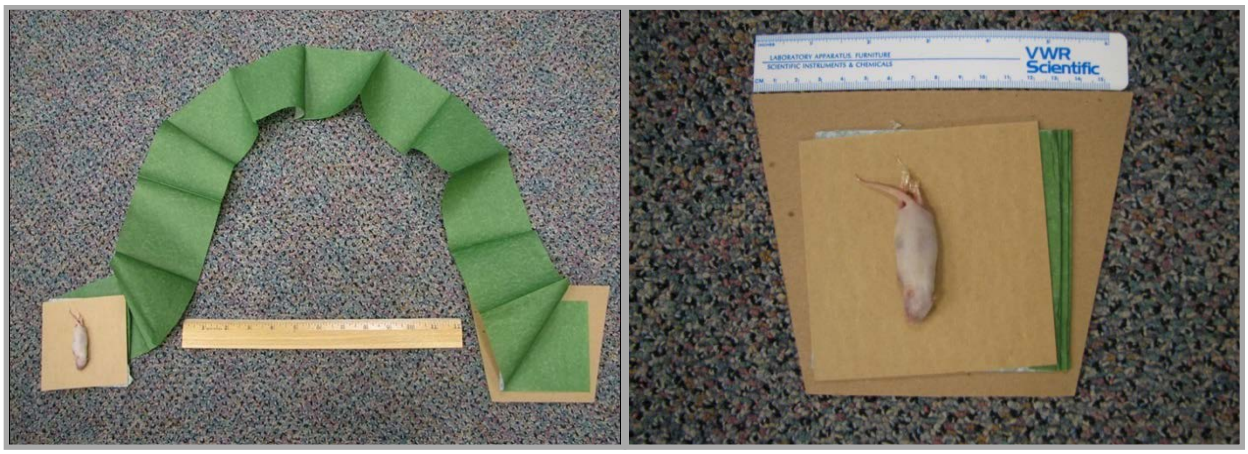
2.0 TECHNOLOGY/METHODOLOGY DESCRIPTION

Technology and methodology was the aerial application of DNM treated with acetaminophen for landscape control of BTS in forests on Guam.

2.1 TECHNOLOGY/METHODOLOGY OVERVIEW

Thawed DNM were treated by inserting an 80 Milligram (mg) acetaminophen tablet into the body cavity through the mouth. Radio transmitters were implanted into the body cavity for tracking a subset of the treated DNM. Treated DNM baits were individually attached to 4 ft. long paper flag streamers with cardboard on each end of the paper streamers (Figure 2). The paper streamer was folded accordion-style between the cardboard, forming a flat, compact flag-bait.

Figure 2. Dead Neonatal Mice Treated with Acetaminophen and Attached to a Flagger and Deployed as Baits for Brown Treesnakes



The flag-baits were packaged into trays and deployed by hand from a helicopter over a prescribed forested drop zone at 36 baits per ha (Figure 3). The double-ended cardboard streamers form a loop in the air and entangle the treated DNM in vegetation above ground level where they can be consumed by the BTS.

Figure 3. Flag-Baits Being Dropped by Hand from a Helicopter over the Forest. Arrow Points to a Flag-Bait that Has Formed an Arc after Being Dropped.

2.1.1 Overall Flow Diagram for the Technology

80 mg acetaminophen tablets formulated at the National Wildlife Research Center (NWRC) → individual tablets inserted into DNM (DNM shipped frozen from the U.S. to Guam and maintained frozen until treated with tablets) → rear legs of DNM hot glued to small piece of cardboard of double-ended cardboard streamer → treated DNM baits stacked in trays and placed in freezer until field deployment → trays loaded into helicopter and baits deployed by hand over forest drop site → radio telemetered DNM (a subset of the total dropped) tracked to determine dispersal pattern and bait take → snake activity on two aerial drop sites and one reference site determined by untreated DNM taken from bait stations and capture of rats in live traps (as snake abundance decreases, rodent abundance may increase).

2.1.2 Chronological Summary of Technology Development

From 1995-1998, the DOD Legacy Program provided funding to the USDA/ NWRC, Fort Collins, Colorado, for development of chemical methods to control BTS. The goal of this project was to identify and develop chemical control methods (oral and dermal toxicants, attractants, repellents, and fumigants) that could be used in an integrated program to control and prevent the dispersal of BTS from Guam and reduce or help control snake populations in various island situations (Engeman and Vice 2001). This project was highly successful and included the identification of formulations of pyrethrins as a dermal toxicant (Brooks et al. 1998a, 1998b), natural products as repellents (Clark and Shivik 2002), and acetaminophen as an oral toxicant (Savarie et al. 2000). From 1999-2003, the DOD Legacy Program funded NWRC for field evaluations of chemicals, bait matrices, and delivery methods for BTS control. During this period, bait station and aerial broadcast field evaluation trials of acetaminophen-treated baits on 6 ha forest plots resulted in 80%-90% reduction of BTS within 4-5 weeks (Savarie et al. 2001, Clark and Savarie 2012). Based on these field trials and other data requirements, acetaminophen was registered by EPA in 2003 as an oral toxicant in DMN baits for BTS control in bait stations and broadcast application by hand, helicopter, and fixed-winged aircraft (EPA Registration Number 56228-34).

From 2001-2007, 7 hand drop aerial delivery studies were conducted on Guam. Two proof-of-concept aerial delivery studies have been conducted. The first, conducted in 2001, deployed non-toxic DNM baits using non-biodegradable plastic streamers and parachutes and demonstrated that baits entangled in the forest canopy were consumed by BTS (Shivik et al. 2002). The second aerial delivery study was conducted in 2002 and was the only study using treated DNM. It showed that after 4 drops totaling 900 - 80 mg acetaminophen-treated DNM on 6 ha of forest, snake activity was reduced by about 80% (Clark and Savarie 2012). A problem with the second aerial delivery was that the corn starch flotation material attached to the DNM dissolved rapidly in the rain and the DNM dropped to the ground. Daily rainstorms are common on Guam and the flotation material must remain intact for 2-3 days before disintegrating. Biodegradable materials were needed to reduce the potential accumulation of litter in the forest. In 2003, five biodegradable flotation materials (paper ring, paper cup, excelsior (wood shavings), burlap, and paper food cup) were evaluated (Savarie and Tope 2004). The highest entanglement in the canopy was only 60% (12 of 20) with the paper food cup.

The DOI/Office of Insular Affairs funded NWRC for bait matrix and aerial delivery development from 2004-2006. In 2004, a study was conducted on low and high crab abundance sites to determine baits taken by non-target animals. Forty radio-telemetered DNM attached to jute mesh and 40 radio-telemetered DNM without jute mesh were deployed. All DNM without jute mesh landed on the ground and 21% of DNM attached to jute mesh landed in the canopy. Comparisons of baits taken between low vs high crab abundance sites was as follows: BTS, 24% vs 0%; crab, 24% vs 67%; monitor lizard, 0% vs 11%; marine toad, 3% vs 0%; ants, 21% vs 14%; unknown, 3% vs 8%; not taken, 26% vs 0% (Savarie et al. 2007). It was evident from these data that a parachute that delivers the majority of the DNM baits to the canopy was needed and that crabs remove mice, making them unavailable for BTS consumption.

In 2005, two small biodegradable parachutes fabricated from paper towels and Ecofilm® (corn derived plastic-like material that decomposes by bacterial degradation) were evaluated, and in 2006, four commercial biodegradable paper products, including single- and double-ender paper streamer marker flags, were tested. Entanglement in the canopy for these 6 products ranged from 67% with paper cups to 95% with the double-ender marker flags (Savarie et al. 2007).

An advantage of the single- and double-ender marker flags was that they can be deployed from an electro-mechanical dispenser mounted on a helicopter. In 2007, the U.S. Navy provided funding for evaluation of single-ender flags. Untreated DNM attached to single-ender flags were deployed from a helicopter by two mechanical dispensers and hand on four ha of forest at 36 baits per ha. There were a total of six drops (three each by dispenser and hand) and 144 flag-baits, including 28 DNM with radio transmitters, were deployed per drop. Canopy landing of the radioed DNM was 85% (61 of 72) by dispenser and 79% (66 of 84) by hand. An important finding from this study was that DNM body fluids from a flag-bait can be transferred to an adjacent flag-bait causing sticking between the 2 flag-baits. This can cause the dispenser to jam and the flag baits are not ejected properly from the dispenser. Another disadvantage of the dispensers was that only 144 flag-baits can be delivered before re-loading. This increases time for deployment. Since several hundred flag-baits can be stored in containers in the helicopter, they were deployed by hand for the demonstration to decrease helicopter time.

2.1.3 Expected Applications of the Technology

The aerial baiting technology deployed on a landscape level in forests will provide the DOD two major benefits for reducing environmental and economic risks caused by BTS. First, it will provide an additional substantial level of defense against snakes getting into outbound cargo by reducing snake populations adjacent to cargo facilities. This will decrease the probability of dispersal of snakes from Guam to vulnerable areas (e.g., Hawaii) where there are no protections to prevent the ecological and economic devastations caused by the snakes on Guam. Secondly, this technology is unlimited in an operational sense. While initially deployed on DOD properties, the ultimate future goal of aerial application is to all inaccessible forests on Guam.

2.2 TECHNOLOGY/METHODOLOGY DEVELOPMENT

Much of the major technology development (e.g. testing of toxicant, bait types, bait delivery methods, and bait stations and monitoring methods) occurred prior to initiation of the Environmental Security Technology Certification Program (ESTCP) project and is covered in Section 2.1.2 above.

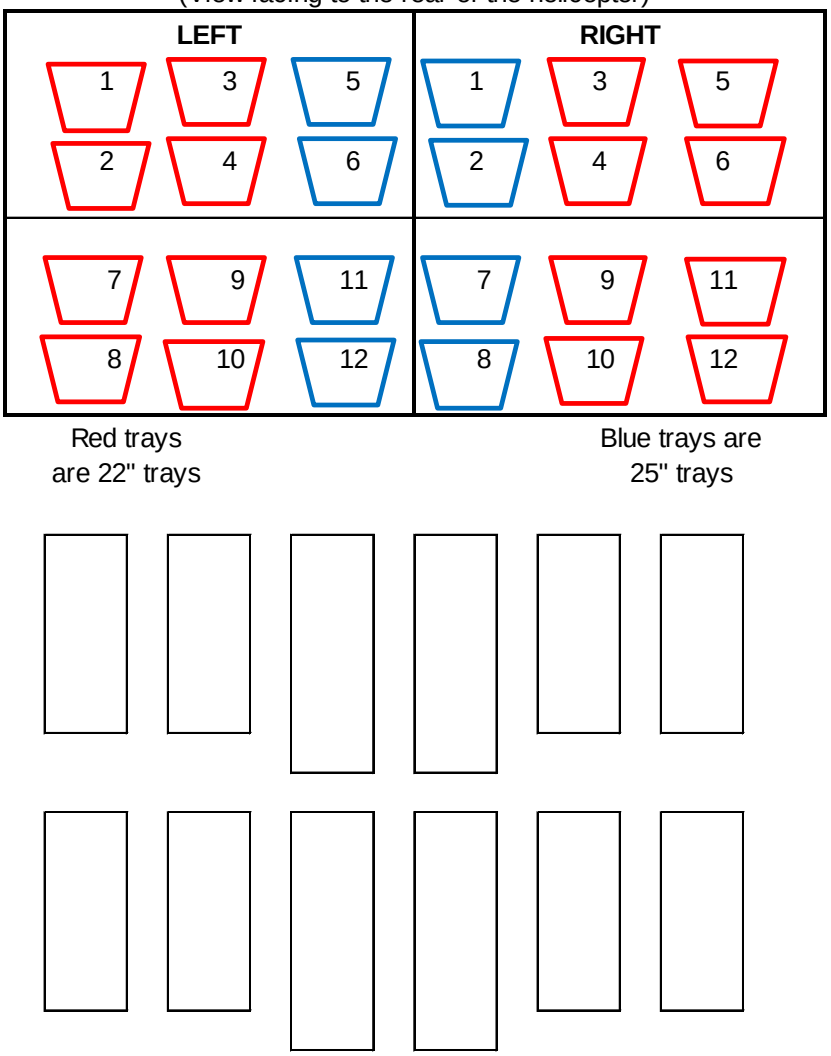
There were some relatively minor modifications to some aspects of the demonstration project that were developed during preliminary trial runs and dictated by situational and operational conditions related to performance; these are discussed below.

Pre-project bait drop trials used a different helicopter and bait handling system and fewer baits. The Bolkow 105 helicopter used in the demonstration project required the development of a flagger bait tray handling system that would allow storage of large numbers of baits in onsite freezers and easy transfer to the cargo area of the Bolkow 105 helicopter. In addition, the tray system needed to be designed to allow identification of specific baits and drop transects by number so randomly selected transect segments could be determined for drops of VHF marked baits. Lastly, in order to allow time for all baits to be dropped on a site and immediate post-drop VHF telemetry tracking to verify bait placement, only one site could be done per day. Each site required two flights to complete a bait drop (i.e. 1,980 baits total). Figure 4 provides an example schematic of flagger bait and tray arrangement for a single bait drop on the HMU site.

Typically Haguruma type traps baited with coconut and with a pre-baiting regime are the preferred methods for Pacific island rodent trapping (Wiewel et al. 2009). However, Haguruma type traps were no longer available necessitating a modification of the standard Tomahawk® type traps to a Haguruma type trigger configuration (Figure 5). The modified and standard traps were compared at an offsite location to evaluate modification performance.

Figure 4. Example Schematic of Flagger Bait and Tray Arrangement for a Single Bait Drop on the HMU Site

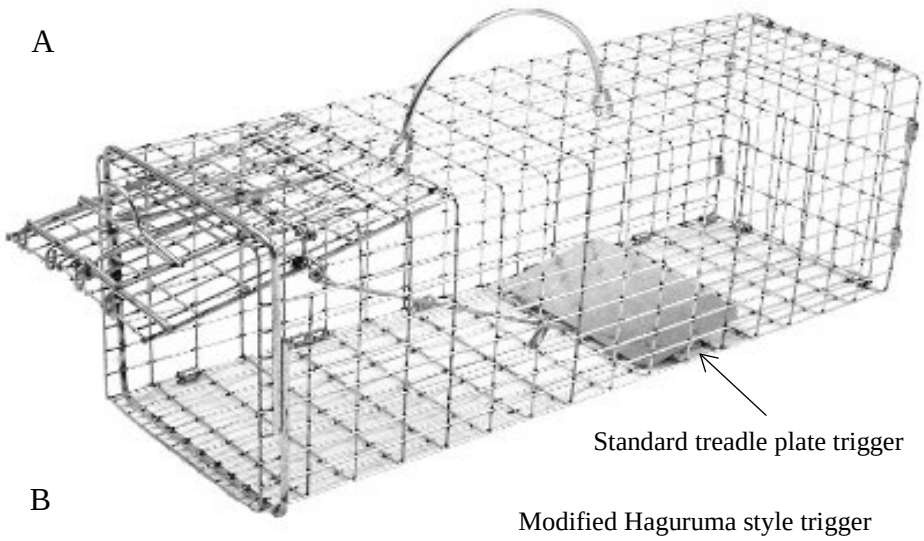
(View facing to the rear of the helicopter)



HMU		Right Side (pilot)	Left Side
Transect	Baits		
1	82	41	41
2	80	40	40
3	80	40	40
4	82	41	41
5	83	41	42
6	83	41	42
7	84	42	42
8	85	42	43
9	86	43	43
10	87	43	44
11	89	44	45
12	91	45	46
Total	1012		

Figure 5. Comparison of standard #33 Tomahawk® rodent live trap (Panel A) and the modified Tomahawk® trap Panel B)

A



B

2.2.1 Advantages and limitations of the technology/ methodology

The primary advantage of the aerial delivery technology is that it can be deployed over inaccessible forests. If snake barriers, such as fences or concrete walls are in place, there would be a permanent reduction in snake population. The limitations of this technology were that flag-baits had to be hand dropped and the high cost of helicopter flight time.

3.0 PERFORMANCE OBJECTIVES

The objective of this demonstration was the efficient deployment of targeted aerial toxicant baits to reduce BTS populations in a large forested landscape. Reduced populations of snakes in areas adjacent to cargo areas may reduce the likelihood of BTS accidently becoming stowaways in outbound cargo from Guam and infesting another vulnerable site. Performance objectives, metrics, data requirements, and success criteria are listed in Table 1 below.

Table 1. Performance Objective Summary Table

Performance Objective	Metric	Data Requirements	Success Criteria	Criteria Met
Quantitative Performance Objectives				
1) Maximize landing of telemetered baits above ground level	Percentage of telemetered baits deployed ground level	Telemetry of radio marked baits above ground	> 80% of telemetered baits deployed above ground level across all drops. Minimum >70% of telemetered baits deployed above ground level per drop	Yes, See section 6.0, p. 33
2) HMU test site: Reduce BTS abundance as measured by reduced telemetered treated DNM bait take by BTS	Percentage of telemetered treated baits taken by BTS after deployment	Number of telemetered treated baits taken by BTS using radio telemetry	No more than four aerial deployments at 2 week intervals for reducing telemetered treated baits taken by BTS by >80%	Yes, See section 6.0, pp. 33-35
3) MSA test site: Reduce BTS abundance as measured by reduced telemetered treated DNM bait take by BTS	Percentage of telemetered treated baits taken by BTS after deployment	Number of telemetered treated baits taken by BTS using radio telemetry	No more than five aerial deployments at 2 week intervals for reducing telemetered treated baits taken by BTS by >80%	No, See section 6.0, pp. 34-35
4) HMU test site: Reduce BTS abundance sufficiently to minimize interval between aerial maintenance deployments	Percentage of untreated DNM baits taken by BTS from bait station transects	Monitor number of untreated DNM baits taken from bait station transects	At least 12 weeks between deployments for maintaining BTS bait take < 30% before next aerial drop	Yes, See section 6.0, pp. 35-41
5) MSA: Reduce BTS abundance sufficiently to minimize interval between aerial maintenance deployments	Percentage of untreated DNM baits taken by BTS from bait station transects	Monitor number of untreated DNM baits taken from bait station transects	At least 4 weeks between deployments for maintaining BTS bait take < 30% before next aerial drop	Yes, See section 6.0, pp. 35-41
6) Minimize compensatory increases in non-native rodent abundance	Index of increased rodent abundance as BTS were removed in HMU and MSA test sites	Number of rats captured from live trap transects in HMU and MSA test sites	< 20 % increase in rodent abundance	Yes, See section 6.0, p. 42
7) Minimize non-target impacts (crabs, monitor lizards)	Number of non-targets identified	Number of radioed baits taken by non-targets	<10% bait take by non-target animals	Yes, See section 6.0, pp. 42-43
8) Maximize aircrew performance for delivering telemetered baits at regular intervals	Uniform interval between telemetered baits after aerial deployment	Radiotelemetry to determine distance of telemetered baits using	Mean range of 17-23 m between telemetered baits	Yes, See section 6.0, p. 43

Table 1. Performance Objective Summary Table (Continued)

Performance Objective	Metric	Data Requirements	Success Criteria	Criteria Met
Qualitative Performance Objective				
1) Maximize aircrew work performance during aerial bait deployment	Ability of aircrew to ward off boredom/ fatigue during bait deployment	Feedback from personnel on effort to deploy baits	Aircrew able to perform duties effectively with minimal boredom/ fatigue	Yes, See section 6.0, p. 43

Quantitative Performance Objectives:

1) Maximize landing of telemetered baits above ground level:

DNM acetaminophen-treated baits were attached to paper streamers with cardboard on each end that entangle the bait above ground level to mitigate bait take by non-target animals such as crabs and monitor lizards. Twenty (20) radio telemetered DNM [10 each on the Habitat Management Unit (HMU) and Munitions Storage Area (MSA)] were deployed at approximately 20 m intervals on each aerial drop on randomly selected 180 m transects along the flight path. Immediately after the drop, DNM were tracked by radio and location (above ground level or ground landing) was recorded. Criterion for success was that the mean for baits above ground level was >80 % across all drops. Minimum performance expected was 70% of telemetered baits above ground level per drop. Six aerial drops were conducted on the HMU and five on the MSA with 10 telemetered baits per drop for a total of 110 telemetered baits dropped.

2) HMU (snake fence barrier aerial test site): Reduce BTS abundance as measured by reduced telemetered treated DNM bait take by BTS

Radio-telemetered DNM were tracked for 1-4 days after each aerial deployment and bait take by BTS and non-target animals was recorded. Trend in snake numbers was measured by a corresponding change in the percentage of radio- telemetered treated DNM consumed by snakes. For example, if bait take was 70% after the first drop and 10% after the fourth drop, the reduction in bait take would be 85.7% (70% - 10% = 60%; 60%/70% = 85.7%). The criterion for success was that no more than four aerial drops were needed for reducing treated bait take by >80%.

3) MSA (no snake fence barrier aerial test site): Reduce BTS abundance as measured by reduced telemetered treated DNM bait take by BTS data acquisition needs were similar to 2) above. The criterion for success was that no more than five aerial drops were needed for reducing bait take by >80%.

4) HMU (snake fence barrier aerial test site): Reduce BTS abundance sufficiently to minimize interval between aerial maintenance deployments. Snake activity was monitored by the number of untreated DNM taken by BTS from bait stations on transects in the HMU. Untreated bait take was expressed as percentage of baits taken. Bait take was recorded pre-aerial drop and approximately every two weeks (weather permitting) for the duration of the study. Aerial drops continued until untreated bait take was < 30%. Aerial drops were initiated again when the bait take was > 30%. The criterion for success was that there was at least 12 weeks between aerial drops for maintaining bait take < 30%.

Amendments to Performance Objective 4: The original Demonstration Plan indicated that bait station monitoring of BTS activity would occur following each bait drop. The plan also implies that some level of interim monitoring would occur but did not specify a frequency for monitoring. The monitoring of bait stations for BTS activity was done every two weeks for the field test portion of the demonstration project. This level of monitoring provided a more rigorous evaluation of post-drop effects and response over time to toxicant bait drops relative to only monitoring immediately post-drop. In addition, monitoring every two weeks provided timely evaluation of the need for further toxicant drops and was logistically feasible for the present project staff level.

The originally planned unadulterated bait station performance metric of a <30% bait take rate was not considered sufficient. At some points in the initial phases of the demonstration the reference site dropped below a 30% bait take rate. Given this, the bait take success rate on the drop sites and the reference site were less than the success metric and therefore a difference from the reference site may not be apparent in some cases. This would hamper evaluation of effectiveness of the method.

Due to the above multiple measures of bait take rate were recommend. One success measure was similar to that proposed for the VHF marked baits. In this case an average > 80% reduction from initial bait takes rates for a given time period. For example, if bait take was 70% after the first drop and 10% after the fourth drop, the reduction in bait take would be 85.7% (70% - 10% = 60%; 60%/70% = 85.7%). In addition, drops were considered successful if overall bait take rates was significantly less ($p<0.05$) on the HMU and MSA as compared to the reference site. This measure allowed the use of the study design and spatial and temporal control to evaluate project success. This method also allowed an evaluation of significant detectable effect sizes (e.g. <30% absolute difference between sites that were detectable at a given alpha) regarding differences between drop sites and the reference site. These changes were applied to both drop sites and were approved in the Fall 2013 ESTCP-IPR and the subsequent white paper.

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5) MSA (no snake fence barrier aerial test site): Reduce BTS abundance sufficiently to minimize interval between aerial maintenance deployments

Data acquisition needs were similar to 4) above. The criterion for success was that there would be at least four weeks between aerial drops for maintaining bait take < 30%, an average > 80% reduction from initial bait takes rates for a given time period, and an evaluation of significant detectable effect sizes (e.g. <30% absolute difference between sites that were detectable at a given alpha) regarding differences between the drop site and the reference site.

6) Minimize compensatory increases in non-native rodent abundance:

It was expected that if snake populations were drastically reduced by the treated baits there would be an increase in rodent abundance. Rodent live traps were used to capture rats from the HMU, MSA and reference sites and cumulative captures throughout the study were recorded. Criterion for success was a <20% increase in rodent abundance across all drops.

<u>Amendments:</u> The modified trapping methods and pre-baiting approved in the Fall 2013 IPR were instituted in an effort to increase trap success as reviewed and. Typically Hagaruma type traps baited with coconut and with a pre-baiting regime are the preferred methods for Pacific island rodent trapping (Wiewel et al. 2009). However, Haguruma type traps were no longer available so the standard Tomahawk® type traps were modified to a Haguruma type trigger configuration. Because of the modification to rodent traps and capture success was low an offsite trap evaluation was set up comparing the modified traps to standard Tomahawk® traps as a check to ensure traps were working effectively.

In the evaluation traps were placed in a systematically random pattern where traps were alternated along transects in an area where *Rattus* species were known to occupy. The traps were then baited and rodents trapped on these transects and the Catch-Per-Unit-Effort (CPUE = the number of rodents caught per 100 trap nights of effort) between trap types was compared using a two sample t-test.

7) Minimize non-target impacts (crabs, monitor lizards)

Radio-telemetered DNM flag-baits entangle in vegetation above the ground to mitigate exposure to non-target animals. Bait take by snakes and non-targets of these telemetered baits was determined by tracking the signal and locating the radio transmitter. The number of baits taken by non-targets were recorded. Criterion for success was <10% bait take by non-target animals across all drops.

8) Determine aircrew performance for delivering radioed baits at regular intervals

Treated baits were deployed at 36 baits per ha, or about one bait every 20 m. To evaluate distribution of baits, 10 telemetered treated baits were included in the 1,980 treated baits dropped per application on each of the two 55 ha aerial drop test sites. The 10 telemetered treated baits were dropped in a randomly selected 180 m straight line transect on the flight path for each aerial application. Immediately after the drop, DNM were tracked by radio and location was recorded. Distance between baits was then calculated from location data. Criterion for success was that the mean distance between baits would be 20 m $\pm$ 3 m all drops.

Qualitative Performance Objectives:

1) Maximize aircrew work performance during aerial bait deployment

It was estimated that would take 4.5-5 hours (h) to deploy the baits on each 55 ha site. Baits were dropped by hand at 20 m intervals on 1,000-1,140 m long flight paths that were spaced 20 m apart. Fatigue could be a problem that would jeopardize safety and performance of the operation. Feedback from the aircrew was assessed to determine if down times after 1-2 h of flight time would be necessary to reduce fatigue.

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4.0 SITE DESCRIPTION

Three 55 ha sites on the northern end of the island of Guam in the western Pacific were selected (Fig. 6). Guam is located at approximately 13 degrees north latitude and 144 degrees east longitude. Guam is about 48 km long and 6 to 19 km wide and has an area of 549 square km. Two aerial drop sites were on Air Force property and the reference site was on Navy property (Figure 6).

Each of the three sites was contiguous with forest habitat that supports BTSs. The two aerial drop sites on Air Force were about 0.7-1.4 km from areas that were being live trapped for snake control. The reference site on Navy was about 1.5 km from snake trapping operations. Habitat on the three sites was characteristic of snake habitat that supports snake populations. Roads were available to access each of the three sites. The distance between sites was not problematic because no assumptions were being made regarding snake abundance. The aerial technique targeted whatever snakes were present.

4.1 SITE LOCATION AND HISTORY

The two aerial drop sites were located on Andersen Air Force Base where munitions were stored and the Reference Site was on the Naval Computer and Telecommunications Station (Figure 6) which provides continuous global and universal communications services to fleet units, shore activities, and joint forces. Andersen Air Force Base provides support to bomber crews deploying overseas, operational support group, search and rescue operations, and Pacific Theater coordination and training opportunities. The terrain on the two drop sites and Reference Site was relatively flat and elevation was about 140 – 153 m (460-500 ft.). The sites were probably disturbed by construction activities during World War II. There were no operations on the two aerial drop sites and the Reference Site that impacted the demonstration.

Access for personnel working on each of the three sites was obtained from the appropriate Air Force and Navy commands. Wildlife Services submitted an Environmental Assessment, "Targeted Aerial Broadcast of Acetaminophen for Brown Treesnake Control on Guam." Final determination for the Environmental Assessment was April 15, 2011. USDA/WS also contacted Guam Department of Aquatic and Wildlife Resources, U.S. Fish and Wildlife Ecological Services, Honolulu, Hawaii, and Air Force and Navy resource managers on Guam regarding this aerial demonstration.

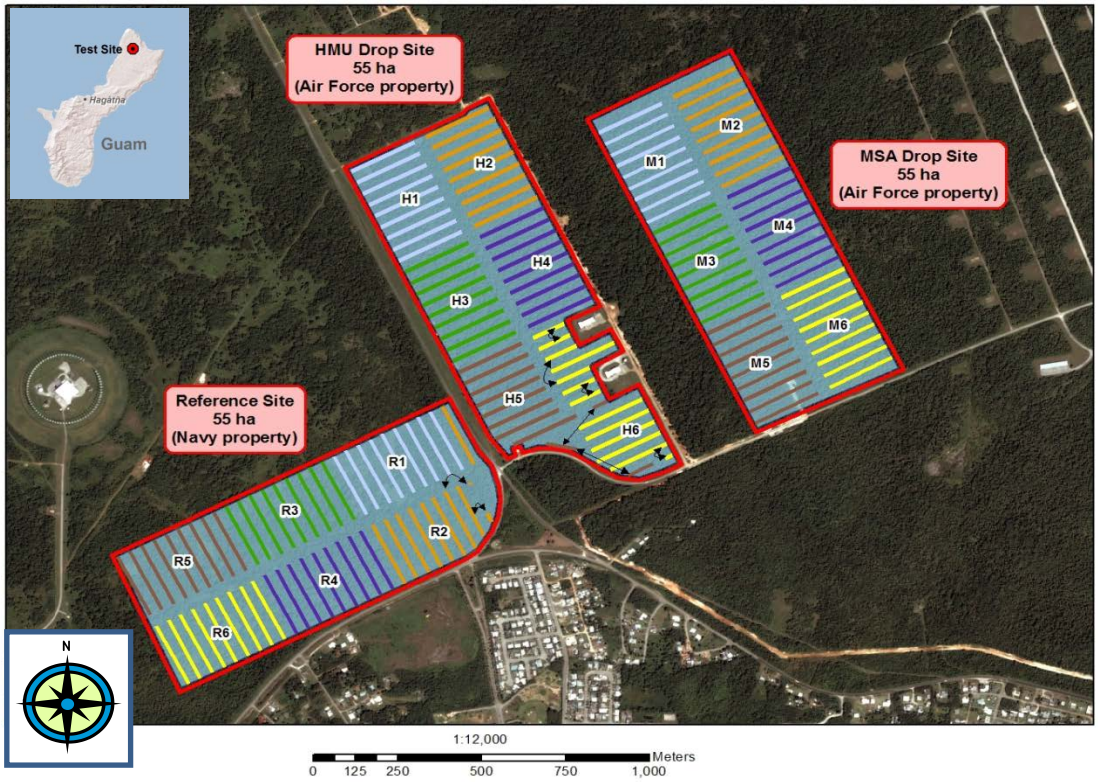
4.2 SITE CHARACTERISTICS

The vegetation is described by the local Chamorro name (**bold**) and the scientific name (*italics*). The three sites were classified as secondary limestone forest, dominated by large, invasive **ahgao manila** (*Vitex parviflora*). Other dominant emergent trees include **nunu** (*Ficus prolixa*), **dukduk** (*Artocarpus mariannensis*), and **yoga** (*Elaeocarpus yoga*), however, **ahgao manila** was by far the most dominant tree. The forest canopy was relatively high, with most areas averaging a height of approximately 15 m. Understory trees include **kaffo'** (*Pandanus tectorius*), **fadang** (*Cycas micronesica*), **fago'** (*Neisosperma oppositfolia*), **paipai** (*Guamia mariannae*), **mapunyao** (*Aglaia mariannensis*), *Eugenia spp.* and **niyok** (*Cocos nucifera*). Herbaceous growth includes **gapit atayaki** (*Wikstroemia spp.*), **tintan-china** (*Cestrum diurnum*), **masikisk** (*Chromolaena odortata*), *Stachytarpheta spp.*, and numerous ferns. There were no conditions that impacted the

demonstration. The climate is characterized as tropical marine. The weather is generally hot and very humid with little seasonal temperature variation. The mean high temperature is 86 °F (30 °C) and mean low is 76 °F (24 °C) with an average annual rainfall of 96 inches (2,438 mm).

Figure 6. Test Sites for Evaluation of the Use of Aerially Applied Acetaminophen-Treated DNM Baits for Control of Brown Treesnakes

Lines in colored quadrants (i.e. M1, M2 etc.) represent 210 m transects on which snake and rodent monitoring was conducted.



5.0 TEST DESIGN

The test design and methods used for this demonstration were variations from previous studies (Savarie et al. 2001, Clark 2003, Savarie et al. 2007) that have been adapted to this demonstration. Sections 5.1 through 5.6 provide detailed review of test design and methods.

5.1 CONCEPTUAL TEST DESIGN

Acetaminophen-treated DNM baits were deployed by hand from a helicopter at the rate of 36 baits per ha on two 55 ha forest aerial test sites (Figure 6). The HMU aerial test site was enclosed by a snake barrier fence to prevent snake immigration into the HMU. The MSA aerial test site and the 55 ha Reference Site do not have barrier fences and both were subject to snake immigration and emigration. The 55 ha forest Reference Site (Figure 6) had the same monitoring procedures as the two aerial test sites, except no treated baits were deployed. Decreased snake populations were monitored by bait take of radio telemetered acetaminophen-treated DNM that were dropped on each of the two aerial sites and bait take of untreated DNM from bait stations located in transects in each of the two aerial sites. Snake activity on the Reference Site was monitored only by bait take of untreated DNM from bait stations located on transects.

5.2 BASELINE CHARACTERIZATION AND PREPARATION

A great amount of resources have been expended in getting the study sites ready for the field test. Using geographic information system global positioning systems (GIS/GPS), each of the two 55 ha aerial test sites and the 55 ha Reference Site have been divided into six blocks of nine transects each (54 transects per site x 3 sites = 162 transects). Each transect has been marked for alternate placement of 11 bait stations at 20 m intervals and 11 rodent live traps at 20 m intervals (54 transects per site x 11 bait station or traps per transect site = 594 possible locations per type). Calculating the total marks for the three sites equals 1,782 possible bait station or rodent live trap locations. Trials to assess BTS breaching the fence into the HMU were not conducted because personnel with fence barrier experience have concluded that the fence meets barrier standards (Perry et al 1998).

5.3 DESIGN AND LAYOUT OF TECHNOLOGY AND METHODOLOGY COMPONENTS

The technology components were: 80 mg acetaminophen tablets (EPA Registration Number 56228-34 - formulated at the NWRC, Fort Collins, Colorado), dead neonatal mice (Noble Supply and Logistics Rockland, Massachusetts), paper streamers (4 ft. long paper flag streamers with cardboard on each end of the paper streamers- R/S Sales, Lewiston, Idaho), radio transmitters (Model 1520, ATS, Isanti, Minnesota), helicopter (Bolkow Bo105), bait stations (5.1 centimeter (cm) diameter x 30.5 cm long polyvinyl chloride tube with a 0.64 cm diameter bolt bisecting each tube 2.5 cm from each end), and rodent live trap (Model 201, Tomahawk® Live Trap, Tomahawk, Wisconsin). Use of trade names does not imply government endorsement.

The 80 mg acetaminophen tablets were formulated at the NWRC as described in Standard Operating Procedure (SOP) Laboratory Procedure (LP) SOP LP 004.00 and shipped to Guam.

DNM were shipped frozen to Guam and maintained frozen until treated with tablets. A tablet was inserted into the body cavity of a thawed DNM through the mouth using forceps. The rear legs and nose of a DNM were glued to the small cardboard on a paper streamer (flag-bait) and flag-baits were re-frozen before being deployed in the field. Flag-baits were deployed by hand at about 30 m above ground level from a helicopter at 36 per ha (1,980 for each aerial drop on each of two 55 ha aerial test sites).

5.4 FIELD TESTING

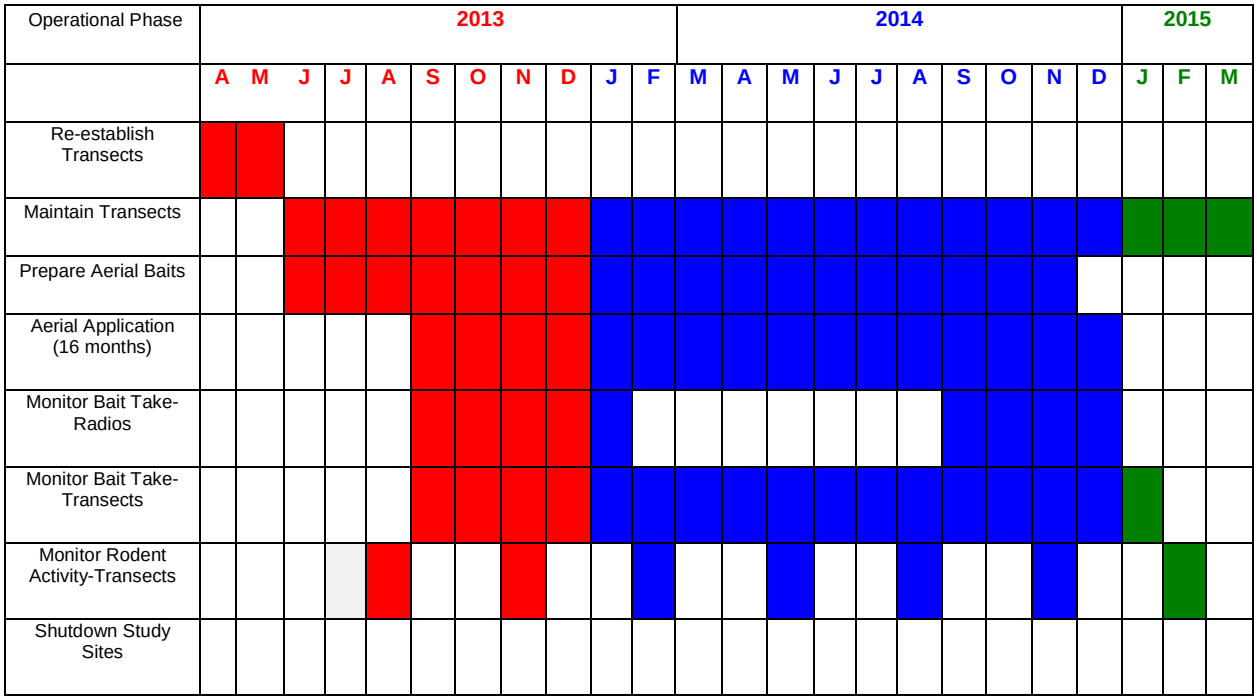
Significant operational phases of the demonstration are presented in the Operational Phase Gantt Chart (Figure 7). Transects in each of the two 55 ha aerial test sites and the 55 ha Reference Site were completed and had to be continually cleared to allow field personnel access. Transects were maintained throughout the 16 months of aerial application. Each aerial drop required 1,980 DNM each treated with an 80 mg tablet of acetaminophen with rear legs and nose glued to paper streamers (flag-baits). Ten of the DNM had radio transmitters for tracking with radio receivers to record the landing position (canopy or ground landing) immediately after the drop, and DNM disposition (take by snakes or non-targets) for 1-4 days post-drop.

Snake activity was monitored by take of untreated DNM bait from bait stations and rodent activity was monitored by capture of rats in live traps (baited with an approximately 1.5 x 1.5 cm segment of coconut) from six transects in each of the two aerial test sites and the Reference Site daily for two days. Transects were randomly selected (ResearchRandomizer, http://www.randomizer.org/form.htm) from each of the six blocks in the two aerial test sites and Reference Site for pre-aerial monitoring and monitoring one week after each aerial drop. Baits missing were not replaced and rats captured were released at the trap site after being individually weighed. Animals found dead on the test sites were collected (SOP FP 034.00).

The HMU and MSA aerial drop sites have two parameters each for scheduling aerial drops after the initial drop on each site: (1) bait take of radio telemetered DNM, and (2) untreated DNM bait take from stations on transects. Bait take of radioed DNM had to be reduced >80% and take of untreated bait from stations <30% before aerial drops were stopped. Thresholds for both parameters on each drop site had to be met to discontinue aerial drops. For example, if radioed DNM bait take was reduced <80% and take of untreated bait from stations was <30% on the MSA site, and both parameters have been met on the HMU site, an aerial drop would be scheduled only on the MSA site.

All physical marks (e.g., plastic flagging and metal markers for transects and marks for bait stations and rodent live traps, as well as the intact bait stations and rodent live traps) were removed at the termination of long term monitoring.

Figure 7. Gantt Chart for Operational Phases of the Aerial Application of Acetaminophen-Treated Baits for Control of Brown Treesnakes



5.5 SAMPLING PROTOCOL

Prior to initiation of the Demonstration Project the NWRC Quality Assurance protocol (QA-1828) was reviewed by the NWRC Quality Assurance Unit (QAU) which is an independent element of the NWRC Director's Office and the research sections of the Center which conduct the studies. The QAU is responsible for monitoring each study to assure that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with the CFR Title 40, Part 160: Good Laboratory Practice Standards (Federal Insecticide, Fungicide, and Rodenticide Act); Title 40, Part 792: Good Laboratory Practice Standards (Toxic Substances Control Act [TSCA]); Title 21, Part 58: Good Laboratory Practice Standards for Nonclinical Laboratory Studies, (Federal Food, Drug, and Cosmetic Act). These duties include appropriate regulated documentation and approval (Institutional Animal Care and Use Committee, Biosafety, National Environmental Policy Act [NEPA], Endangered Species Act [ESA]) as applicable.

All data collection followed the Demonstration Plan protocol (see sections 3.0 and 5.0) and NWRC QA-1828. In addition data collection followed NWRC SOP AD011.02 Data Recording and Error Correction. Field data sheets identifying NWRC QA-1828 were developed for specific field data collection activities (e.g. bait station monitoring, rodent trapping). Details on calibration, quality assurance, methods, and number and type of samples collected are provided in the following sections including referenced figures and appendices.

Prior to aerial bait drops a subsample of acetaminophen tablets were sent to the NRWC chemistry Unit labs in Ft. Collins, CO, for assay of quality and amount of active ingredient. This procedure followed NWRC Analytical Method 96B -Determination of Acetaminophen in Tablets.

Brown Treesnake and non-target animal carcasses were shipped to the NWRC chemistry labs along with control samples collected offsite through normal WS operational programs. All samples were single blind evaluated by the chemistry lab staff to ensure accuracy of findings. Standard operating procedures for analyses followed NWRC Analytical Method 106A - Determination of Acetaminophen Residues in Brown Treesnake Carcasses.

Field data recording and entry were checked for accuracy by third party review, typically a support staff member, onsite supervisor, or the principal investigator. Rodent trapping and rodent trap modifications were evaluated by offsite trapping and comparison of the modified traps used in this study to standard Tomahawk® traps. This comparison was conducted to ensure that the modified rodent traps were performing as designed to meet project data collection needs. Control of bias was addressed by having a Reference Site that was monitored by bait station monitoring and rodent trapping using the same procedures as the two aerial drop sites (HMU and MSA), except it was not treated with toxicant baits.

Field data were recorded onsite on hardcopy data sheets at the time of data collection and were scanned and archived at the WS, NWRC Quality Assurance Unit archive in Ft. Collins, CO.

5.6 SAMPLING RESULTS

The following provide the quantitative performance objectives results and data summary of Very High Frequency (VHF) radio telemetry data collected for the following performance metrics:

1) Maximize landing of telemetered baits above ground level

2) HMU test site: Reduce BTS abundance as measured by reduced telemetered treated DNM bait take by BTS

3) MSA test site: Reduce BTS abundance as measured by reduced telemetered treated DNM bait take by BTS

4) Minimize non-target impacts (crabs, monitor lizards)

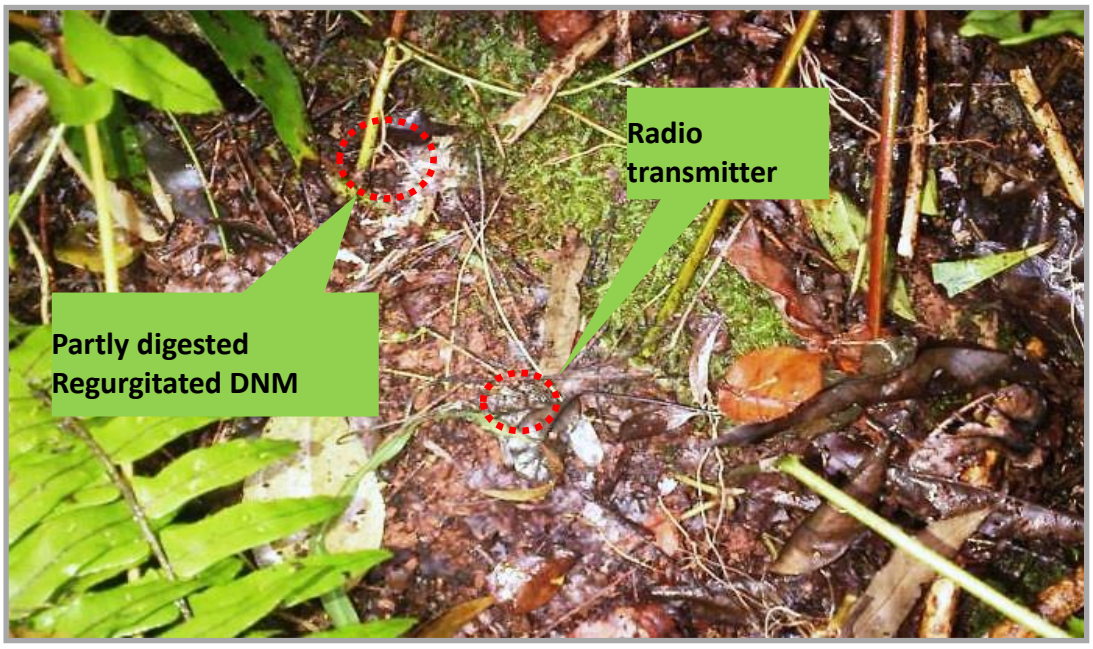
5) Maximize aircrew performance for delivering telemetered baits at regular intervals.

A total of 110 telemetered baits were dropped over 11 applications. Of these it was possible to make a determination of canopy or ground landing, distance between baits, and bait take rates on each site for 105 telemetered baits that were recovered in the field (95% recovery rate). One radio was never relocated, 1 was not deployed (it fell into the interior of the helicopter) and 3 were not categorized due to delays in recovery due to weather. The radio transmitter's batteries began to fail rapidly after 14 months and further data could not be collected.

While metrics for quantitative performance objectives one and eight for telemetered baits worked well, achieving metrics quantitative performance objectives two and three presented challenges in

field application. The issue was that snakes were taking the baits but a large number appeared to regurgitate the VHF transmitter, prior to mortality. So, while 95% of VHF transmitters were recovered, determination of fate and take rate was difficult as transmitters were not found in snakes. In order to address the presumed regurgitation of transmitters, only baits identified in the canopy immediately post-drop and later recovered on or near the ground and "clean" (i.e. not attached to a mouse) were used in analyses as an indicator of take rate. In some cases the radio was found near the carcass of a snake or regurgitated parts of a mouse (Figure 8).

Figure 8. Image of a Regurgitated Mouse and VHF Transmitter Found during Transmitter Recovery Efforts on the MSA Drop Site, Andersen Air Force Base, Guam, 4 September 2013



Due to the lack of VHF telemetry based bait fate data, carcasses of BTS found on each drop site were opportunistically collected (HMU n=6, MSA n=6) during bait-station monitoring and site maintenance. An additional eight snakes collected from outside the bait drop areas were sent (single blind) to the USDA/WS/NWRC lab in Ft. Collins, Colorado, for acetaminophen residue analyses. Standard operating procedures for analyses followed NWRC Analytical Method 106A - Determination of Acetaminophen Residues in Brown Treesnake Carcasses.

The following provide the quantitative performance objectives results and data summary of bait drop and bait station data collected for the following performance metrics:

1) HMU test site: Reduce BTS abundance sufficiently to minimize interval between aerial maintenance deployments
2) MSA (no snake fence barrier aerial test site): Reduce BTS abundance sufficiently to minimize interval between aerial maintenance deployments

Figure 9 provides a schematic example of aerial bait drop, bait station and rodent trapping methods on the HMU site. The MSA and reference site followed similar procedures except the reference site had no aerial bait drops. The blue boundary line represents the snake barrier fence. The parallel lines running the length of the site represent the aerial toxicant bait drop transects. Toxicant baits were dropped approximately every 20 m. The red dots represent the randomly selected aerial transect segment on which VHF marked baits were dropped. The white lines perpendicular to the aerial transects represent the 210 m long bait station monitoring transects or rodent monitoring transects randomly selected for each monitoring period. Each transect had 11 bait stations or rodent traps placed at 20 m intervals.

Figure 9. Schematic Example of Aerial Bait Drop, Bait Station and Rodent Trapping Methods on the HMU Site

A total of 29,700 80 mg acetaminophen tablets inserted in DNM and attached to a flagger were aerially deployed on the 55 ha HMU and MSA bait drop sites during 15 applications between 11 September 2013 and 19 December 2014 (Table 2).

Table 2. Drop Date, Consecutive Bait Drop Number on Each Drop Site and Number of 80 mg Acetaminophen Tablets Deployed during Each Drop on the HMU and MSA Drop Sites

Andersen Air Force Base, Guam, 3 September 2013 to 19 December 2014.

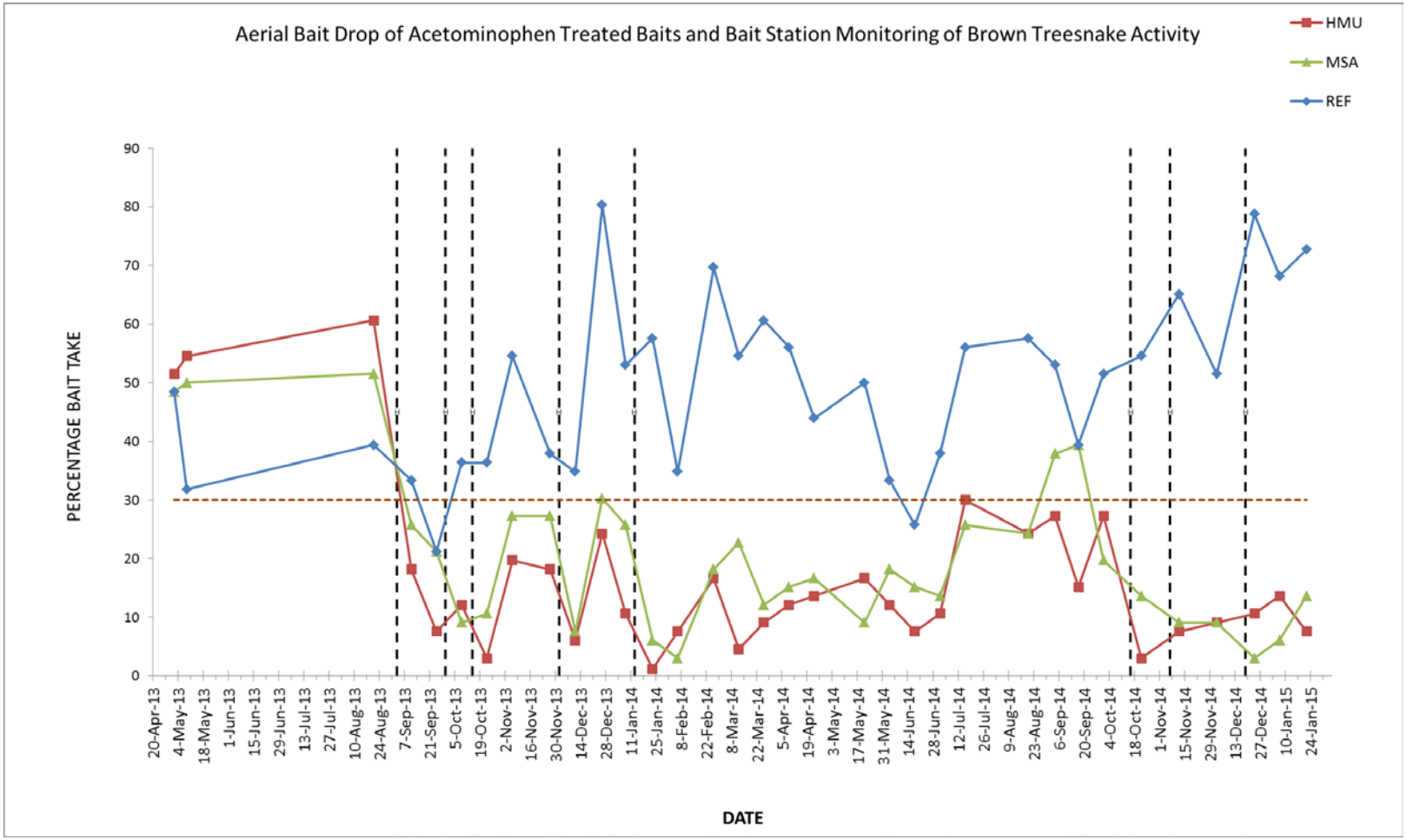
HMU			MSA		
Drop Date	**Drop Number**	**80 mg Acetaminophen Tablets Deployed**	**Drop Date**	**Drop Number**	**80 mg Acetaminophen Tablets Deployed**
3-Sep-13	1	1,980	4-Sep-13	1	1,980
30-Sep-13	2	1,980	1-Oct-13	2	1,980
15-Oct-13	3	1,980	16-Oct-13	3[a]	0
2-Dec-13	4	1,980	3-Dec-13	4	1,980
13-Jan-14	5	1,980	14-Jan-14	5	1,980
15-Oct-14	6	1,980	16-Oct-14	6	1,980
6-Nov-14	7	1,980	7-Nov-14	7	1,980
19-Dec-14	8	1,980	19-Dec-14	8	1,980
Total		15,840	Total		13,860

[a]Drop on HMU only, severe weather prevented drop on MSA site

A total of 6,732 bait stations were baited and checked, providing 612 individual transect level measures of bait take rate over 34 monitoring sessions from 2 May 2013 to 22 January 2015 (Figure 10).

Figure 10. Bait Take Rate from Bait Stations on Randomly Selected Transects on the Reference Site (REF, blue solid line, Naval Station, Guam), HMU Drop Site (Solid Red Line, Andersen Airforce Base, Guam) and MSA (Solid Green Line, Andersen Airforce Base, Guam) 2 May 2013 through 22 January 2015

Vertical black dashed lines represent bait drop dates on the HMU and MSA. The red horizontal dashed line was the 30% reference level below which bait take rates should be maintained for each drop site.



The following provide the quantitative performance objective results and data summary for rodent trapping data collected for the following performance metric:

1) Minimize compensatory increases in non-native rodent abundance.

A total of five rats (likely Rattus diardii: Species status on Guam is in question (Wiewel et al. 2009). Hereafter all rats are referred to as Rattus spp.) were captured in 2,772 total trap nights of sampling on all three locations. All five roof rats were captured on the HMU location, one on 13 August 2013 prior to bait drops, one on 12 August 2014, and three on 25 and 26 November 2014.

Modified trap test performance data: A total of 12 rats (Rattus spp.) were caught in 420 trap nights of effort (210 per trap type). The average CPUE was 3.8 rodents/100 trap nights and 1.9 rodents per 100 trap nights for the modified and standard traps respectively.

The following provides the qualitative performance objective data summary for maximizing crew performance for the following performance metric:

1) Maximize aircrew work performance during aerial bait deployment**.**

The aircrew's success in this qualitative metric was measured by the successful completion of demonstration project activities. Crews maintained production levels and enthusiasm for all aspects and phases of the demonstration project evidenced by: 1) study site establishment of 162, 210 m transects (34 km total), placement of 1,782 bait stations on transects, 2) preparation of 29,700 flagger baits, 3) radio tracking and recovery of 105 VHF marked baits, 4) 15 total aerial bait drops of 1,980 baits each (29,700 total), 5) twice monthly baiting and monitoring of 198 randomly selected bait stations over 34 sessions (6,732 bait stations total), and 6) 2,772 total trap nights of rodent sampling on all three locations.

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6.0 PERFORMANCE ASSESSMENT

Quantitative Performance Objectives:

1) Maximize landing of telemetered baits above ground level

Results:

Of the 105 recovered baits, 86 were found in the canopy, providing an 82% canopy landing of baits over all sites and all drops (min=50%, max=100%). Canopy landing of baits was marginally greater in the HMU than the MSA (mean = 85% and mean = 79%, respectively). This was largely driven by a low landing rate of 50% in drop two for the MSA. The median canopy landing rate for the HMU and MSA was 85% and 88% respectively. A F-test of canopy landing data indicated equal group variances ($F_{5,4}$ = 0.58, P=0.19). A two sample t-test assuming equal variances indicated no significant difference (t_9 = 0.60, p=0.54) between sites. The mean height in the canopy of baits was 11.2 m (N=105, SD=6.13m).

Summary Conclusions Canopy Landing of Baits:

The success criterion for > 80% landing of baits in the canopy was met. The use of VHF transmitters placed in the body cavity of DNM baits worked well for tracking bait landing locations and evaluating success metrics. Major factors in bait landing success were the presence of wind and rain during and immediately post-drops. Wind not only affected drift of the flaggers and baits, it also made it more difficult for the pilot to stay on transects. Avoiding applications when winds might exceed 15 kilometers per hour would improve performance. Conducting bait drops during rain events were avoided for the most part, however, on at least one occasion rain occurred within 48 h post-drop (drop 4). The rain caused the flaggers to disintegrate causing some baits to fall to the ground.

2) HMU (snake fence barrier aerial test site): Reduce BTS abundance as measured by reduced treated telemetered DNM bait take by BTS.

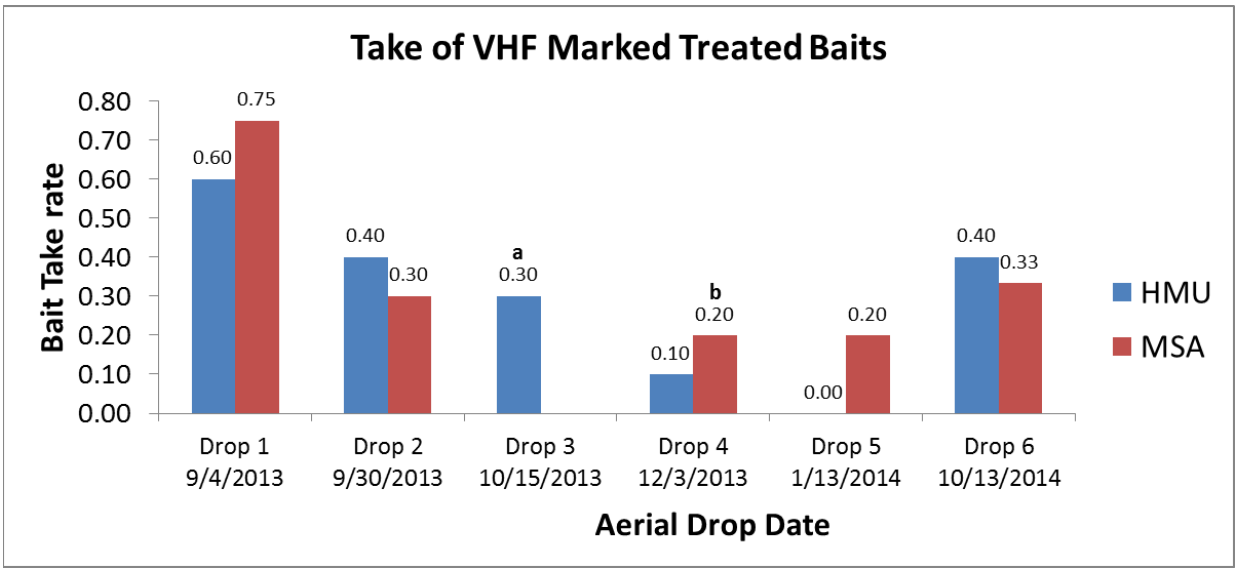
Analysis of data:

Descriptive statistics for calculated percentage change (reduction) in treated bait take from initial drop for each monitoring period.

HMU:

For bait drops 1-5 on the HMU site there was a decline in bait take rate of VHF marked treated baits. Bait take rate on the HMU site declined from 60% to 10% by bait drop 4 (Figure 11). There was an 83% decline in bait take rate of treated VHF marked baits on the HMU, thus reaching the success metric of an 80% decline within 4 drops (Table 3). When bait drops resumed almost 10 months later bait take rates on the MSA had increased to levels similar to those recorded during the 2nd bait drop but did not increase to initial bait take levels suggesting a potential long-term reduction in bait take rate and consequently snake numbers on the HMU site.

Figure 11. Bait Take Rate of VHF Telemetered Treated Baits Dropped on Random Transects on the HMU and MSA Drop Sites, Andersen Air Force Base, Guam, 4 September 2013 – 13 October 2014 (N=110)



[a]Drop on HMU only, severe weather prevented drop on MSA site

[b]Heavy rains >24 hours post-drop caused some flaggers on the HMU to fall to the ground possibly affecting snakes bait take rates.

Table 3. Reduction in Bait Take Rate Relative to Bait Take Rate at the First Aerial Bait Drop on the HMU and MSA Drop Sites

Andersen Air Force Base, Guam, 4 September 2013 – 13 October 2014. Bait take rate determined from 10 VHF marked toxicant baits (N=110 total) aerially dropped on randomly selected transects on each drop site for each bait drop date.

Aerial Drop Site	Drop 1 9/4/2013 N=10 per site	Drop 2 9/30/2013 N=10 per site	Drop 3 10/15/2013 N=10	Drop 4 12/3/2013 N=10 per site	Drop 5 1/13/2014 N=10 per site	Drop 6 10/13/2014 N=10 per site
HMU Bait Take Rate	0.60	0.33	0.50	0.83	1.00	0.33
MSA Bait Take Rate	0.75	0.60	a	0.73	0.73	0.56

[a]Drop on HMU only, severe weather prevented drop on MSA site

3) MSA (no snake fence barrier aerial test site): Reduce BTS abundance as measured by reduced treated telemetered DNM bait take by BTS

Analysis of data: Descriptive statistics for calculated percentage change (reduction) in treated bait take from initial drop.

MSA: For bait drops on the MSA there was a decline in bait take rate of VHF marked treated baits after the first five drops (Figure 11). Bait take rate on the MSA site declined from 75% to 20% by bait drop five (Figure 11). There was a 73% decline in bait take rate of treated VHF marked baits on the MSA (Table 3). However, on the MSA site it was not possible to meet the success metric of an 80% decline. Due to bad weather, the MSA only had four bait applications over this time period total. When bait drops resumed almost 10 months later, bait take rates on the MSA had increased to levels similar to those recorded during the 2nd bait drop but did not increase to initial levels suggesting a potential long term reduction in bait take rate and consequently snake numbers on the MSA site.

Summary Conclusions MSA and HMU VHF Marked Treated Baits:

Bait take rate of VHF telemetered baits clearly did decline on both the HMU and MSA sites. The success metric of a >80% reduction in bait take rate within 4 drops on the HMU was achieved. Although the MSA did not reach the target level of an 80% reduction in bait take rate over 5 drops, extenuating factors of one drop being cancelled due to severe weather and the possibility of overestimation in bait takes rate may have negatively affected results. The data for the MSA site clearly indicated a decline in snake numbers and reached a 73% reduction in 4 applications which is marginally successful.

A total of 14 BTS were sent (single blind) to the USDA/WS/NWRC lab in Ft. Collins, Colorado, for acetaminophen residue analyses. For snakes recovered within drop sites, 83% (N=6) tested positive for residual acetaminophen. No snakes (N=8) recovered outside of the drop sites were positive for residual acetaminophen. These results suggest that snakes dying within drop sites were doing so largely due to consumption of acetaminophen treated baits.

4) HMU (snake fence barrier aerial test site): Reduce BTS abundance sufficiently to minimize interval between aerial maintenance deployments

Analysis of data: The observations that were analyzed from each transect (one per each of the six sampling blocks for each of the two aerial drop sites and the reference site) were the proportion of baits taken from the bait stations. Data were tested using a general linear model (Hocking 1985, SAS Institute 2011) two-factor factorial analysis of variance, where main effects were treatment location (REF, HMU, MSA) and time (sampling occasion). Treatments were also compared for bait drop periods and sampling occasions as determined by success metrics using *a priori* contrasts on the treatment x time interaction means (Hayter 1989). Data were analyzed using least squares means and Tukey-Kramer adjustment for P-values for multiple comparisons (SAS Institute 2011).

Analyses Results: The general linear model for pre- and post-drop bait takes rate was significant ($F_{17, 396} = 27.34$, $P < 0.001$). Main effects drop period, location and the drop period by location interaction were significant ($F_{5, 396} = 23.10$, $P < 0.001$, $F_{2, 396} = 120.00$, $P < 0.001$, and $F_{10, 396} = 10.93$, $P < 0.001$, respectively). The Tukey-Kramer adjustment for means comparisons was performed on all pairwise differences in means for all locations pre-drop and post-drop for bait drops 1-5. All bait drop sites were significantly different than the reference site during drop periods 2-5 (Table 4). Means comparisons indicated that the bait take rate within the HMU declined significantly with respect to pre-drop take of unadulterated mouse baits from bait stations after single aerial toxicant bait drop (Figure 12). The HMU drop site was significantly less than the reference site for drop periods 1-5. Both drop sites were not significantly different from each other for drop periods 1-5. The HMU had a significantly greater bait take rate than the reference site pre-drop (Figure 13).

Figure 12. Bait Take Rate (%) Within Drop Sites and Reference Site Pre- and Post-Drop after a Single Toxicant Bait Application

Vertical bars represent standard deviations. Asterisks (***) represent significant differences using Tukey-Kramer post-hoc comparisons at α = 0.05. NS indicates no significant difference. Minimum detectable effect size for changes in bait take rate for the HMU, MSA, and Reference (REF) site were, 12.2%, 10.3% and 12.6% respectively.

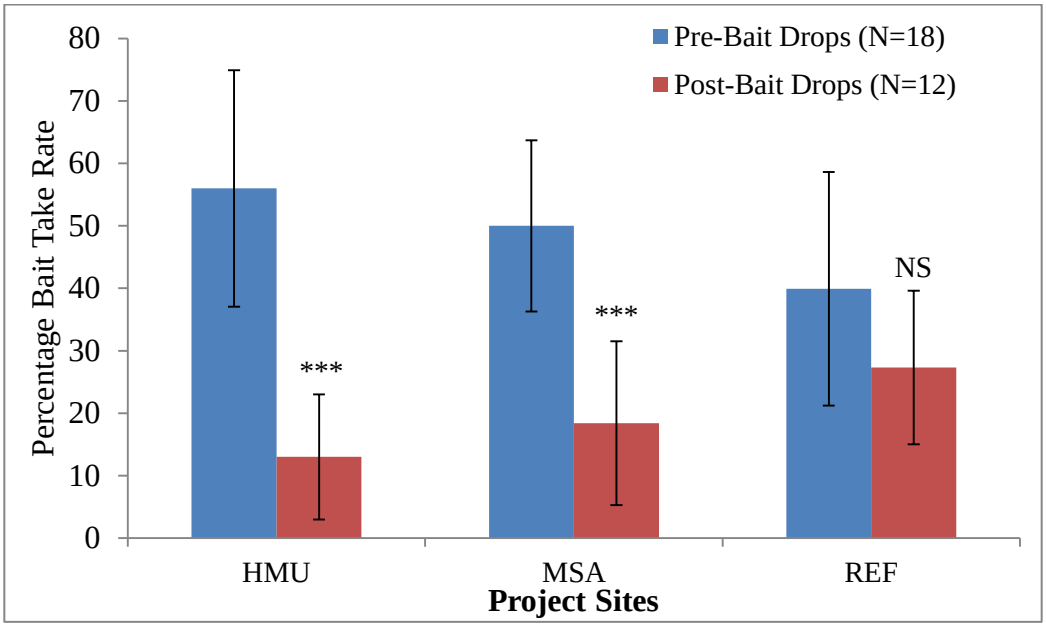


Figure 13. Comparison of Pre-and Post-Drop Bait Take Rates after a Single Bait Application between Drop Sites and Reference Site (May 02, 2013-September 23, 2013)

Letters represent significant differences between locations pre- and post-drop using the Tukey-Kramer post-hoc means comparison and α =0.05. Locations with the same letter were not significantly different.

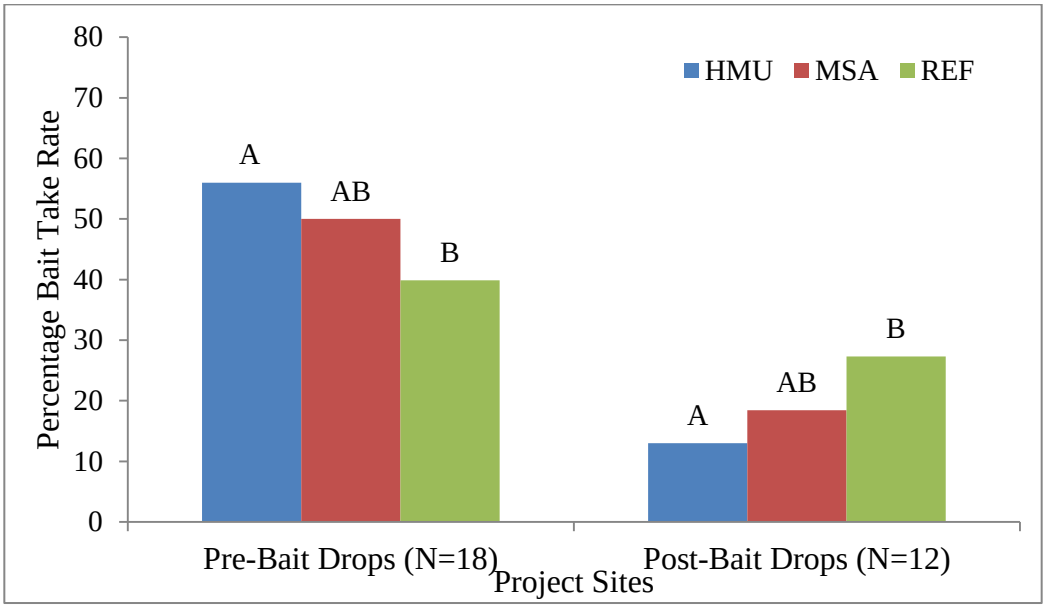


Table 4. Comparisons for all pairwise differences between bait take rate means for pre-drop and all bait drop periods and locations for bait drops 1-5 for aerially applied acetaminophen treated baits to control Brown Treesnakes

The upper cell for each comparison is t for H_0: LSMean(i)=LSMean(j). The lower cell in each comparison is the Pr > |t|.

		MSA	REF	HMU	MSA	REF	HMU	MSA	REF	HMU	MSA	REF	HMU	MSA	REF	HMU	MSA	REF
	i/j	**Pre-drop**	**Pre-drop**	**Drop 1**	**Drop 1**	**Drop 1**	**Drop 2**	**Drop 2**	**Drop 2**	**Drop 3**	**Drop 3**	**Drop 3**	**Drop 4**	**Drop 4**	**Drop 4**	**Drop 5**	**Drop 5**	**Drop 5**
HMU	**Pre-**	1.04	2.93	7.15	5.37	4.74	5.75	6.16	2.54	7.85	6.34	2.37	7.85	6.43	-0.09	10.65	9.84	1.85
	drop	1.00	0.24	<.01	<.01	0.00	<.01	<.01	0.50	<.01	<.01	0.63	<.01	<.01	1.00	<.01	<.01	0.93
MSA	**Pre-**		1.89	6.22	4.44	3.81	5.02	5.42	1.81	6.81	5.30	1.32	6.81	5.39	-1.14	9.35	8.54	0.55
	drop		0.91	<.01	0.00	0.02	0.00	<.01	0.94	<.01	<.01	1.00	<.01	<.01	1.00	<.01	<.01	1.00
REF	**Pre-**			4.53	2.75	2.12	3.68	4.08	0.47	4.92	3.41	-0.57	4.92	3.50	-3.03	6.98	6.17	-1.82
	drop			<0.01	0.35	0.81	0.03	0.01	1.00	0.00	0.07	1.00	0.00	0.05	0.19	<.01	<.01	0.94
HMU	**Drop**				-1.62	-2.20	0.09	0.47	-2.93	-0.13	-1.48	-5.04	-0.13	-1.40	-7.24	0.53	-0.15	-6.92
	1				0.98	0.75	1.00	1.00	0.24	1.00	0.99	0.00	1.00	1.00	<.01	1.00	1.00	<.01
MSA	**Drop**					-0.58	1.42	1.80	-1.61	1.65	0.30	-3.26	1.65	0.38	-5.46	2.64	1.96	-4.81
	1					1.00	0.99	0.94	0.98	0.97	1.00	0.11	0.97	1.00	<.01	0.42	0.89	0.00
REF	**Drop**						1.89	2.27	-1.14	2.28	0.93	-2.62	2.28	1.02	-4.82	3.40	2.71	-4.06
	1						0.91	0.70	1.00	0.69	1.00	0.44	0.69	1.00	0.00	0.07	0.37	0.01
HMU	**Drop**							0.33	-2.62	-0.20	-1.27	-4.08	-0.20	-1.20	-5.82	0.28	-0.22	-5.20
	2							1.00	0.44	1.00	1.00	0.01	1.00	1.00	<.01	1.00	1.00	<.01
MSA	**Drop**								-2.95	-0.60	-1.67	-4.48	-0.60	-1.61	-6.22	-0.16	-0.67	-5.65
	2								0.23	1.00	0.97	0.00	1.00	0.98	<.01	1.00	1.00	<.01
REF	**Drop**									3.01	1.94	-0.87	3.01	2.01	-2.61	3.83	3.33	-1.65
	2									0.20	0.89	1.00	0.20	0.86	0.45	0.02	0.09	0.97
HMU	**Drop**										-1.51	-5.49	0.00	-1.42	-7.95	0.81	0.00	-7.99
	3										0.99	<.01	1.00	0.99	<.01	1.00	1.00	<.01
MSA	**Drop**											-3.97	1.51	0.09	-6.43	2.71	1.90	-6.09
	3											0.01	0.99	1.00	<.01	0.38	0.91	<.01
REF	**Drop**												5.49	4.07	-2.46	7.69	6.88	-1.11
	3												<.01	0.01	0.56	<.01	<.01	1.00
HMU	**Drop**													-1.42	-7.95	0.81	0.00	-7.99
	4													0.99	<.01	1.00	1.00	<.01
MSA	**Drop**														-6.53	2.59	1.78	-6.21
	4														<.01	0.47	0.95	<.01
REF	**Drop**															10.77	9.96	1.97
	4															<.01	<.01	0.88
HMU	**Drop**																-1.24	-13.44
	5																1.00	<.01
MSA	**Drop**																	-12.20
	5																	<.01

There was no significant increase in bait take rate within the HMU between bait drop five (monitoring session 13, 23-January-2014) until monitoring session 24 (16-July-2014; a period of 160 days without a significant detectable increase in bait takes rate on the HMU (Figure 10). During this period the HMU averaged a bait take rate of 10.2% (N=66, SD=4.8%, min=0, max=36.4%) corresponding to an average 81.7% (N=66, SD= 8.7%) reduction in bait take rate.

Bait take rates increased significantly between monitoring sessions 23 (11%) and 24 (30%) and reached the 30% threshold on the HMU for the first time since 21 August 2013; a period of 315 days (Figure 10). The general linear model for within the HMU site bait takes rate for session 23 and 24 was significant ($F_{1,\ 10} = 13.85$, $P = 0.004$) The HMU had a significantly higher bait take rate in session 24 (Δ=19%, minimum detectable difference = 11.8%, at α=0.05 and β=0.80). Based on these factors a decision was made to initiate a second series of bait drops on the HMU.

For the second series of bait drops the pre-drop period was considered the point at which either the HMU or MSA exceeded the 30% threshold or had a significant increase in bait take rates. Given the data the 2nd pre-drop period began on 16 July 2014 (session 24) and continued to 1 October 2014 (session 28). Three consecutive bait drops were conducted from 16 October to 19 December 2014. Bait stations were then monitored until 22 January 2015 (Figure 10).

<u>Analyses Results 2nd Bait Drop Period:</u>

The general linear model for pre- and post-drop bait takes rate was significant ($F_{11,\ 186} = 36.68$, $P < 0.001$). Main effects drop period, location and the drop period by location interaction were all significant ($F_{3,\ 186}= 5.41$, $P=0.001$, $F_{2,\ 186}= 143.99$, $P < 0.001$, and $F_{6,\ 186}= 3.78$, $P < 0.001$, respectively).

There was not a significant drop in bait take rate pre- and post-bait drops 6-8 within the HMU although there were marginal reductions ($P < 0.10$ Table 5). However, the HMU drop site was significantly less than the reference site pre-drop and for drop periods 6-8 (Table 5). The HMU was not significantly different from the MSA pre-bait drop or for bait drop periods 6-8 (Table 5).

There was no significant increase in bait take rate within the HMU site post bait drop eight; a period of 29 days without a significant detectable increase (Figure 10). During this period the HMU averaged a bait take rate of 8.6% (N=36, SD=8.7%, min=0, max=27.3%) corresponding to an average 84.5% (N=36, SD= 6.4%) reduction in bait take rate.

5) MSA (no snake fence barrier aerial test site); reduce BTS abundance sufficiently to minimize interval between aerial maintenance deployments

Data acquisition needs were similar to 4) above. The criterion for success was that there would be at least four weeks between aerial drops for maintaining bait take < 30%. <u>Analysis of data:</u> The observations that were analyzed from each transect (one per each of the six sampling blocks for each of the two aerial drop sites and the reference site) were the proportion of baits taken from the bait stations. The analytical structure was a two-factor factorial analysis of variance, where one factor was treatment and the other was time (sampling occasion). Treatments were compared for each sampling occasion using *a priori* contrasts on the treatment x time interaction means.

The general linear model for pre- and post-drop bait takes rate between drop sites and reference site and drop periods 1-5 was significant ($F_{17, 396} = 27.34$, $P < 0.001$). The Tukey-Kramer adjustment for means comparisons was performed on all pairwise differences in means for all locations pre-drop and post-drop for bait drops 1-5. The bait take rate within the MSA declined significantly with respect to pre-drop take of unadulterated mouse baits from bait stations after single aerial toxicant bait drop (Figure 12). Bait take rate on the MSA was significantly different than the reference site during drop periods 2-5 (Table 4) but not drop period 1 (Figure 13). The bait take rate on the MSA was intermediate between the HMU and reference site pre and post-drop, but not significantly different from either after one toxicant bait application. Both drop sites were not significantly different from each other for drop periods 1-5.

There was no significant increase in bait take rate within the MSA site between bait drop five (monitoring session 13, 23-January-2014) until monitoring session 26 (4-Sep-2014; a period of 224 days without a significant detectable increase in bait take rates on the MSA (Figure 10). During this period the MSA averaged a bait take rate of 15.4% (N=78, SD=13.5%, min=0, max=54.5%) corresponding to an average 69.2% (N=78, SD= 13.5%) reduction in bait take rate.

Bait take rates increased significantly between monitoring sessions 23 (14%) and 26 (38%) and reached the 30% threshold on the MSA for the first time since 26 December, 2013; a period of 252 days (Figure 10). The general linear model for within MSA bait take rate for session 23 and 26 was significant ($F_{1, 10} = 14.88$, $P = 0.003$). The MSA had a significantly higher bait take rate in session 26 (Δ=24%, minimum detectable difference = 14.0%, at α=0.05 and β=0.80). Based on these factors a decision was made to initiate a second series of bait drops.

For the second series of bait drops the pre-drop period was considered the point at which either the HMU or MSA exceeded the 30% threshold or had a significant increase in bait take rates. Given the data the 2nd pre-drop period began on 16 July 2014 (session 24) and continued to 1 October 2014 (session 28). Three consecutive bait drops were conducted from 16 October to 19 December 2014. Bait stations were then monitored until 22 January 2015 (Figure 10).

<u>Analyses Results 2nd Bait Drop Period:</u>

The general linear model for pre- and post-drop bait takes rate was significant ($F_{11, 186} = 36.68$, $P < 0.001$). Main effects drop period, location and the drop period by location interaction were all significant ($F_{3, 186}= 5.41$, $P=0.001$, $F_{2, 186}= 143.99$, $P < 0.001$, and $F_{6, 186}= 3.78$, $P < 0.001$, respectively).

There was a significant drop in bait take rate pre- and post-bait drops within the MSA for bait drops periods 7 and 8 (Table 5). The MSA drop site was significantly less than the reference site pre-drop and for drop periods 6-8 (Table 5). The MSA was not significantly different from the HMU pre-bait drop or for bait drop periods 6-8 (Table 5).

There was no significant increase in bait take rate within the MSA site post bait drop eight; a period of 29 days without a significant detectable increase in bait take rates on the MSA (Figure 10). During this period the MSA averaged a bait take rate of 9.1% (N=36, SD=10.0%, min=0, max=36.4%) corresponding to an average 81.8% (N=36, SD= 8.4%) reduction in bait take rate from pre-drop rates.

Summary Conclusions for Bait Station Monitoring on All Sites:

Bait take rate of unadulterated baits placed in bait stations indicated significant and sustained reductions in bait take rates after aerial application of acetaminophen treated DNM. There were significant drops in bait take rates after a single application of 1,980 baits within both the HMU and MSA drop sites and between both drop sites and the reference site (Figures 12 &13). These reduced bait take rates were sustained for over 160 days on the HMU site with an average reduction in bait take rate of 81.7%. The bait take rate on the HMU site did not exceed a 30% bait take rate after the first bait drop for a period of 315 days. These metrics easily surpassed the original success metrics of maintaining bait take rate below 30% on the HMU for 84 days (12 weeks) post-drop, although five drops were made on the HMU site to achieve this result.

During all drop periods both the HMU and MSA had significant reductions in bait take rate relative to the reference site and relative to within site pre-drop bait take rates. The MSA site exceeded the 80% reduction threshold in bait take rates for 34 days post-drops. There was no significant increase in bait take rate within the MSA site for a period of 224 days with an average reduction in bait take rate of 69.2% over this time period. In addition, bait take rates did not increase above the 30% threshold on the MSA for a period of 252 days easily exceeding the original success metric of at least 28 days below a 30% bait take rate. It should be noted that this was achieved after only four bait drops as the 3rd bait drop was cancelled due to severe weather.

As was seen with telemetered baits, bait take rate from unadulterated baits in bait stations clearly did decline on both the HMU and MSA sites. The success metric of a >80% reduction in bait take rate on the HMU was achieved and this average reduction was sustained for over five months. The MSA reached the target level of an 80% reduction in bait take rate and did so in four aerial bait drops and was sustained for more than four weeks. Longer-term reductions in bait take rate were on average less than the HMU but not significantly so. In addition there was not a significant increase in bait take rate on the MSA for over seven months and the bait take rate did not exceed 30% for over eight months. Bait station monitoring indices of BTS abundance clearly show a significant decrease in snake numbers which corroborates findings from VHF marked baits. Original success metrics of maintaining bait take rates below 30% on both the HMU and MSA for 12 and 4 weeks, respectively were achieved. Long-term bait take reductions were also achieved and these were significantly less than the reference site for all drop periods. Original success metrics were met for both sites and more stringent success metrics applied after the Fall 2013 IPR were met on the HMU and largely met on the MSA.

Table 5. Comparisons for all Pairwise Differences between Bait Take Rate Means for Pre-Drop and All Bait Drop Periods and Locations for Bait Drops 6-8 for Aerially Applied Acetaminophen Treated Baits to Control Brown Treesnakes

The pre-drop period began 16 July 2014 (session 24) and continued to 1 October 2014 (session 28). The upper cell for each comparison is t for H_0: LSMean(i)=LSMean(j). The lower cell in each comparison is the Pr > |t|.

		MSA	REF	HMU	MSA	REF	HMU	MSA	REF	HMU	MSA	REF
	i/j	**Pre-drop**	**Pre-drop**	**Drop 6**	**Drop 6**	**Drop 6**	**Drop 7**	**Drop 7**	**Drop 7**	**Drop 8**	**Drop 8**	**Drop 8**
HMU	**Pre-drop**	-1.153	-6.765	3.196	1.642	-4.350	3.167	3.022	-6.422	3.129	3.795	-10.630
		0.992	<.001	0.069	0.891	0.001	0.075	0.111	<.001	0.083	0.011	<.001
MSA	**Pre-drop**		-5.612	3.862	2.308	-3.684	4.039	3.894	-5.550	4.128	4.794	-9.632
			<.001	0.008	0.475	0.015	0.004	0.007	<.0001	0.003	<0.001	<.0001
REF	**Pre-drop**			7.102	5.548	-0.444	8.281	8.136	-1.308	8.988	9.654	-4.771
				<.001	<.001	1.000	<.001	<.001	0.977	<.001	<.001	<0.001
HMU	**Drop 6**				-1.203	-5.845	-0.695	-0.794	-7.245	-1.053	-0.632	-9.755
					0.988	<.0001	1.000	1.000	<.0001	0.996	1.000	<.0001
MSA	**Drop 6**					-4.641	0.695	0.596	-5.856	0.421	0.842	-8.281
						0.001	1.000	1.000	<.001	1.000	1.000	<.001
REF	**Drop 6**						6.054	5.955	-0.496	6.106	6.527	-2.597
							<.001	<.001	1.000	<.001	<.001	0.289
HMU	**Drop 7**							-0.122	-8.023	-0.399	0.133	-11.407
								1.000	<.001	1.000	1.000	<.001
MSA	**Drop 7**								-7.901	-0.266	0.266	-11.274
									<.001	1.000	1.000	<.001
REF	**Drop 7**									8.389	8.922	-2.619
										<.001	<.001	0.277
HMU	**Drop 8**										0.596	-12.307
											1.000	<.001
MSA	**Drop 8**											-12.902
												<.001

6) Minimize compensatory increases in non-native rodent abundance

Analysis of data: The observations that were analyzed from each transect (one per each of the six sampling blocks for each of the two aerial drop sites and the reference site) were the number of rats captured per trap-night. The analytical structure was a two-factor factorial analysis of variance, where one factor was treatment and the other was time (sampling occasion). Treatments were compared for each sampling occasion using *a priori* contrasts on the treatment x time interaction means.

Analyses Results:

Treatment Sites: A total of five rats (likely *Rattus diardii*: Species status on Guam is in question (Wiewel et al. 2009). Hereafter, all rats are referred to as *Rattus spp*.) were captured in 2,772 total trap nights of sampling on all 3 locations. All five roof rats were captured on the HMU location, one on 13 August 2013 prior to bait drops, one on 12 August 2014, and three on 25 and 26 November 2014. Rodent live trapping results were not statistically analyzed as too few rodents were caught on any of the demonstration project sites over the demonstration period to make valid analyses.

Modified Trap Test Results: There was no significant difference in CPUE between modified or standard Tomahawk® traps in the offsite evaluation of modified traps. A total of 12 Rattus spp. were caught in 420 trap nights of effort (210 per trap type). The average CPUE was 3.8 rodents/100 trap nights and 1.9 rodents per 100 trap nights for the modified and standard traps respectively.

Summary conclusions for Rodent Trapping:

The CPUE's for offsite trapping were much greater than that observed for onsite trapping. Applying the offsite CPUE for the modified traps to the demonstration project drop sites would result in an estimated capture of 106 rodents on the demonstration sites. These results indicate the modified traps worked at least as well as standard traps and were functioning adequately to measure real changes in rodent numbers. Therefore it is concluded that over the demonstration project period there has been no significant compensatory increase in rodent numbers due to a reduction in BTS associated with aerial bait drops.

7) Minimize non-target impacts (crabs, monitor lizards)

Telemetered DNM flag-baits entangle in vegetation above the ground to mitigate exposure to non-target animals. Bait take by snakes and non-targets of these telemetered baits were determined by tracking the signal and locating the radio transmitter. The numbers of baits taken by non-targets were recorded. Criterion for success was <10% bait take by non-target animals. Analysis of data: Descriptive statistics for calculated percentage of non-target animals that take telemetered bait across all drops.

Analyses Results:

Of the 105 recovered VHF marked toxicant baits only one bait was found in a non-target species; a cane toad (*Rhinella marina*). The cane toad was found on the HMU site on 3 December 2013. However, while checking bait stations one juvenile monitor lizard was found dead on the HMU drop site.

The monitor lizard was tested for residual acetaminophen and tested positive indicating it did die from consumption of one or more baits. Counting the monitor lizard there was a < 1.9% non-target bait take rate relative to the number of VHF marked baits deployed.

Summary Conclusions for Minimize Non-Target Impacts (crabs, monitor lizards):

Only one non-target was found to have consumed a VHF marked toxicant bait of the 105 deployed and recovered. An additional non-target was discovered opportunistically and tested and found to have been exposed to acetaminophen baits. One of these non-targets was an invasive cane toad and the other was a naturalized juvenile monitor lizard. Even counting both of these species the success metric of a < 10% non-target bait take rate was successfully met.

8) Maximize aircrew performance for delivering radioed baits at regular intervals

GPS coordinates were used to determine distance between the radioed baits. Analysis of data: Descriptive statistics were performed across all drops.

Analyses Results:

The mean distance between telemetered baits dropped on randomly selected aerial transects was 19.3 m (N=96, SD = 16.6 m, Min= 1.0 m, Max = 86.2).

Summary Conclusions for Bait Delivery.

The bait delivery success metric of a mean distance between baits of 20 m $\pm$ 3 m was successfully met. It should be noted that weather (wind and rain) can dramatically affect bait delivery and performance as evidence by the relatively high standard deviation in bait drop locations. Selection of days to apply baits must take weather into consideration for the entire drop period for bait delivery to be successful.

Qualitative Performance Objective

1) Maximize aircrew work performance during aerial bait deployment

The aircrew's success in this qualitative metric was demonstrated by the successful completion of demonstration project activities. Crews maintained production levels and enthusiasm for all aspects and phases of the demonstration project including: 1) study site establishment of 162, 210 m transects (34 km total), placement of 1,782 bait stations on transects, 2) preparation of 29,700 flagger baits, 3) radio tracking and recovery of 105 VHF marked baits, 4) 15 total aerial bait drops of 1,980 baits each (29,700 total), 5) twice monthly baiting and monitoring of 198 randomly selected bait stations over 34 sessions (6,732 bait stations total), 6) 2,772 total trap nights of rodent sampling on all 3 locations, and 7) transect and snake barrier maintenance.

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7.0 COST ASSESSMENT

7.1 COST MODEL

Table 6. Cost Model Based on the Cost Estimates in Section 7.3 above Broken Down to a Cost Per ha Basis

Each section represents operational phases and cost per ha for each phase.

Cost Model for Aerial Delivery Technology for a 500 ha site

Cost Element	Data Tracked During the Demonstration	Estimated Cost per ha
Site preparation	Labor cost per hour for delineating area of test sites and transects for monitoring and costs of non-consumable supplies and equipment	$672.33
Bait preparation	Labor cost per hour, costs of consumable supplies and rate of use	$932.91
Aerial Bait deployment	Cost per hour and total hours for rental helicopter and labor for aircrew and flight safety personnel and costs of non-consumable supplies and equipment	$1,624.56
Monitoring	Cost for snake and rodent abundance monitoring and costs of non-consumable supplies and equipment	$2,245.92
Total Cost per ha	Total costs for all the above	$5475.72

7.2 COST DRIVERS

The assessment of cost drivers is based on demonstration project costs and potential issues associated with scaling up to a 500 ha operational program. The assessment is broken down into operational phases in the following order: 1) Aerial site preparation, 2) Aerial bait preparation, 3) Aerial bait deployment, 4) Brown tree snake abundance monitoring, 5) Rodent abundance monitoring.

Aerial drop site preparation – Scaling up to 500 ha would provide an estimated cost of $336,166 ($672/ha) to establish 490 monitoring transects at approximately 1-210 m transect per ha. Most (73%) of this cost was associated with labor for field employees.

Aerial bait preparation – Cost of bait preparation was largely driven by cost of baits, flaggers (39% of the total cost) and labor to prepare baits (35% of total costs; see). At an application rate of 36/ha it would require 144,000 baits to conduct eight applications over a one year period. Based on results of the demonstration project this application rate should be sufficient to keep snake numbers at an 80% or more reduction from pre-drop rates.

Aerial bait deployment – Cost of aerial helicopter serves was by far the largest cost associated with this operational phase. Aerial operations cost comprised 64% of this operational phase total costs. There were considerable unexpected costs of helicopter services over the demonstration project.

The contractor initially did not indicate that the Bolkow 105 helicopter was not on site and ferry costs would be incurred over the course of the project. After the first drop the service provider refused to fly unless this ferry cost was incorporated as well as a 4 h minimum flight time. As they were the only provider on Guam this essentially would shut down the project. Although some costs were negotiated down ultimately helicopter costs exceeded planned costs. Costs per application for each site averaged $3,584/h were as much as $5,619/h (min = $3,105/h). Costs per deployment were on average $13,573 for each site equating to an average of $5,027 per hour for the actual average deployment time of 2.7 h per 55 ha site. This cost per hour greatly exceeded the planned $2,950/h and $3,150/h for FY13 and FY14 respectively.

Monitoring – Monitoring includes assessing bait take of telemetered treated bait by snakes and non-target animals, and BTS abundance using bait take of untreated DNM in bait stations and capture of rodents in live traps. Monitoring to evaluate effects of aerial bait drops on snake numbers would be an essential component of any operational brown tree snake control program and is also the most labor intensive aspect of a control program overall. Total labor costs for snake and rodent monitoring account for 71% of total costs for this phase of an operational program.

Total Costs – Labor costs were the single largest driver of operational costs and account for 48% of total costs (see Table 7). The second largest single component was aerial operations costs which accounted for 19% of total costs. If bait and flagger costs are included the total is 25%. Methods to reduce labor costs, particularly associated with monitoring, and aerial operation costs would be logical focus areas for cost control in an operational program.

7.3 COST ANALYSES AND COMPARISON

The following cost analyses and comparison is based on demonstration project costs scaled up to a 500 ha operational program. The cost assessment is broken down into operational phases in the following order: 1) Aerial site preparation, 2) Aerial bait preparation, 3) Aerial bait deployment, 4) Brown tree snake abundance monitoring, 5 Rodent abundance monitoring, 6) Total cost.

Aerial site preparation – For calculating area (ha) of the site and establishing transects for monitoring snake and rodent abundance. Personnel costs per hour, total time, and payroll records, as well as consumable supplies such as flagging were tracked. The life-cycle cost estimate was per annum and scaling was from 110 ha [the two 55 ha aerial drop sites (HMU and MSA)] to 500 ha. The demonstration has 9 monitoring transects per 9.2 ha [each of the two 55 ha aerial drop sites were divided into six blocks and each block (9.2 ha) has nine transects] and transect density per ha could be reduced for implementation sites. The decrease in transect density has not been determined. However, labor costs may not be reduced due to the much larger size of the implementation sites and accessibility to these transects. Annual salary increases will increase the cost estimate.

Cost for initial establishment of transects on the 2-55 ha drop sites was $70,263 ($639/ha), during FY 2013. This included costs for one supervisory staff, ong GIS staff, admin, and five field staff measuring and clearing N=108, 210 m transects (i.e. 54 on each site), access to transect locations, vehicle fuel, supplies, and overhead. Because rodent trapping was done on the same transects as bait station monitoring for snake bait take rate, a separate estimate for rodent monitoring transect establishment was not necessary.

Scaling up to 500 ha would provide an estimated cost of $336,166 ($672/ha) to establish 490 monitoring transects at approximately 1-210 m transect per ha. Most of this cost was associated with labor for field employees. Subsequent maintenance costs for transects are not included in this estimate. It should be noted that this estimate assumes that all areas of a 500 ha drop site would be as accessible as the demonstration project sites. This may not be the case and more difficult access could add substantially to costs.

The intensity of monitoring necessary to fully evaluate the aerial acetaminophen bait drop technology may not be necessary or feasible for subsequent larger scale operations applications. Density of one transect per ha could be reduced or placement of transects could be in representative but smaller monitoring areas (e.g. random 10 ha plots) within the overall drop zone. Altering monitoring strategies should be carefully evaluated prior to implementation but could be a potential source of substantial cost savings in subsequent transect establishment, maintenance and monitoring at larger operational scales.

Aerial bait preparation – For preparing acetaminophen-treated DNM baits with and without radio transmitters for aerial test sites. Personnel costs per hour, total time, and payroll records were tracked in addition to acetaminophen tablets, DNM, paper streamer flags, and radio transmitters that are consumable supplies. The life-cycle cost estimate was per annum and scaled from 110 ha to 500 ha.

Cost of bait preparation was largely driven by cost of baits, flaggers and labor to prepare baits.

At an application rate of 36/ha it would require 144,000 baits to conduct eight applications over a one year period. Based on results of the demonstration project this application rate should be sufficient to keep snake numbers at an 80% or more reduction from pre-drop rates. Given this information, the total annual cost of aerial bait preparation and tracking equipment is estimated at $466,457.

Figure 14. Preparation of Acetaminophen Treated DNM for Aerial Application to Control Brown Treesnakes

Images are of manual insertion of 80 mg acetaminophen tablets into DNM, attachment to flaggers, and storage in metal boxes which were then placed in a freezer until date of application.

Aerial bait deployment – Treated baits were deployed using a contracted helicopter/pilot and cost per hour and total time were tracked. Additionally, labor cost per hour and total time for aircrew to maintain flight paths via computer and drop baits, landing zone manager for maintaining radio contact (required by USDA flight safety regulations), and ground based personnel were tracked.

Non-consumable safety equipment for the aircrew was tracked. Except for safety equipment, the life-cycle cost estimate was per annum and scaling from 110 ha to 500 ha. Safety equipment costs were annuitized.

Figure 15. Bolkow 105 Helicopter and Crew in Preparation for Aerial Deployment of DNM Treated with an 80 mg Acetaminophen Tablet to Control Brown Treesnakes

Racks in image to the right contain DNM baits attached to flaggers.

There are several factors that will influence costs per hour for scaled up estimates. It is unlikely that the 4 hour minimum would be a factor as flights times will exceed the minimum for a 500 ha drop site. Ferry time would be a factor assuming the same contractor is used and was included in subsequent cost estimates. Applications appeared to be more efficient toward the end of the demonstration project declining from an average of 2.8 h to 2.6 hours per 55 ha site (min=2.1 h). Not having to differentiate between bait sites and locations and running longer transects with less time for turning may also make deployments more efficient. It may also be possible to increase the size of the bait rack on the helicopter and add additional baits, reducing the number of trips to reload. Based on these factors it was assumed that baits could be applied to 250 ha per eight h flight for the resulting budget of $812,281.

Scaling up to operational BTS control programs will likely have other economies of scale. For example larger contracts may encourage additional flight services to bid at lower cost per hour flight times. In addition, USDA, WS has their own aviation program with both fixed and rotary wing aircraft. It is likely that the USDA, WS cost of flights would be substantially less than the current private contractor cost estimates on a cost per hour basis. However, getting a helicopter to Guam would likely incur considerable cost and would not be justified for USDA except for larger areas ($\geq$ 500 ha) and multi-year contracts. Lastly, development of automated bait delivery technology may further increase aerial application efficiency.

<u>Budget Estimate Using Automated Bait Delivery Device Technology:</u>

USDA, WS, NWRC researchers are currently developing an automated bait delivery device to increase efficiency of aerial deployment of toxicant baits to control Brown Treesnakes. The automated system has several characteristics which could increase efficiency of bait drops and reduce costs. Primary cost savings with this technology are: 1) only one aircrew member is needed versus three with hand delivery, 2) air speed is increased at least two-fold over hand delivery, 3) bait

deployment rate is up to 4/ second, or 14,400 per hour; so a 500 ha site could theoretically be baited in 1.25 hours, 4) device capacity of 3,600 baits/load vs 990 for manual application. The currently used Bolkow 105 helicopter could potentially carry up to 7,200 baits requiring only 2.5 reloads on a 500 ha site. Expected production cost of DNM baits is about $1.00 per bait. Current hand baiting technology allows for about 1,000 baits per flight. Given these potential increases in bait delivery efficiency an estimated one day of delivery at 3,600 baits per load and one day of preparation for bait drops was used. The total estimated cost for bait delivery on a 500 ha site using the automated bait delivery technology is estimated at $450,975, a 44% cost savings over the current technology. These estimates are theoretical as the technology has not yet been field tested.

Monitoring – Monitoring includes assessing bait take of telemetered treated bait by snakes and non-target animals, and BTS abundance using bait take of untreated DNM in bait stations and capture of rodents in live traps. Personnel costs per hour, total time, consumable supply costs of DNM and non-consumable supply costs of plastic bait stations and rodent live traps were tracked. Except for non-consumable supply costs, the life-cycle cost estimate was per annum and scaling from 110 ha to 500 ha. Non-consumable supply costs were annuitized.

Monitoring to evaluate effects of aerial bait drops on snake numbers would be an essential component of any operational brown tree snake control program. Monitoring is also the most labor intensive aspect of a control program overall. Scaling up from the demonstration project and maintaining the same level of monitoring effort would require the establishment of 490, 210m transects, with 11 bait stations on each transect, from which 54 (594 bait stations) would be randomly selected twice monthly for baiting, monitoring and evaluation of bait take rates. To prevent entrainment of snakes to bait stations which may artificially increase bait take rates, random selection of transect is critical. The budget for bait station monitoring of $849,593 reflects this level of monitoring.

It may be possible to reduce the number of transects and possibly the frequency (e.g. once every 3-4 weeks) and still maintain the ability to detect changes in snake numbers on a 500 ha site. For example it may be possible to reduce the total number of transects to 162 and randomly select 54 transects from that group. This would reduce up-front costs of transect establishment, reduce the number of bait tubes and transect maintenance, and reduce monitoring effort, potentially reducing costs substantially.

Although there was no measurable increase in rodent numbers on the drop sites over the demonstration project period, the need for monitoring rodent's remains. The 16 months of monitoring on the demonstration project may not have been long enough to allow establishment of a rodent population. As with bait station monitoring, labor is a large component of the cost of rodent monitoring. Rodent monitoring should be conducted on the same transects as bait stations saving some start-up cost. Given a proportional effort as the demonstration project's rodent monitoring, a 500 ha drop site would require monitoring of 54 transects with 11 traps on each transect (594 traps) which would be monitored for two days (1,188 trap nights) each session, and pre-baited with coconut for <72 hours prior to trap set. Monitoring would occur quarterly and transects would be randomly selected in each monitoring period. Unlike the demonstration project, all rodents trapped in an operational program would likely be euthanized as they are an invasive species. The budget estimate of $273,364 was based on the aforementioned level of rodent trap monitoring.

As with bait station monitoring, it may be possible to reduce the number of transects and still maintain the ability to detect changes in rodent numbers on a 500 ha site. Since monitoring of rodent traps and bait stations occurs on the same transects changes to one monitoring effort would affect the other and this should be considered with respect to operational design. A similar reduction in costs as bait station monitoring could be achieved by reducing the number of transects and total number of traps checked.

Equipment procurement – For maintaining helicopter on prescribed flight paths and delineating test sites and transect lines. Formal and on-the-job training were required for personnel to be proficient in instruments for maintaining helicopter flight paths and GIS/GPS of study sites, as well data collection. Invoices for all equipment purchases and training were tracked. Field life of this equipment is estimated to be 5-8 years. The equipment and training estimates were annuitized so it can be extrapolated over a longer period.

Equipment purchases have been annuitized over a 5 year service life. Annuitized equipment costs on an annual basis for a 500 ha bait drop operational program would be $42,731. This includes costs for VHF transmitters and receivers although it should be noted that only receivers are annuitized as transmitters have about a 1 year life. Annuitized equipment costs are included in the above estimates for each breakdown of the operational aerial bait drop program on a 500 ha site.

Vehicles – Vehicles were used for transporting personnel and supplies to the aerial test sites. Purchase price, fuel, and maintenance record costs were tracked. Field life of the vehicles was estimated to be 5-8 years. The vehicle cost was annuitized so it can be extrapolated over a longer period.

Initial vehicle purchase costs are substantial ($210,000 for seven vehicles) but for a longer term operational program. The annuitized cost of the estimated 7 vehicles needed to support field crews for bait station monitoring, rodent monitoring, VHF tracking, site maintenance and aerial operations support would amount to $26,250 -$42,000 per year based on a 5-8 year operational life. Annual costs including the annuitized vehicle cost, fuel, and maintenance amount to $38,048-$60,877 per year based on a 5-8 year operational life. Annuitized vehicle costs are included in the above estimates for each breakdown of the operational aerial bait drop program on a 500 ha site.

Overall Operational Cost for a 500 ha Control Site:

The estimated budget to aerially apply DMN baits with an internal 80 mg acetaminophen tablet by hand over a 500 ha site including monitoring of brown tree snake numbers, rodent trapping and monitoring, and VHF tracking for determination of bait fate is estimated at $2,737,860 in FY15 dollars (Table 7).

The demonstration project cost $1,876,430 to complete including the research component. Given that a 500 ha site is 3.03 times the area of the all demonstration project sites combined and 4.54 times the area of the demonstration project drops sites, there may be economies of scale cost reductions for an operational program relative to the demonstration project. A strict scale up from the 165 ha demonstration site not including the NWRC research component would result in a conservative operational cost of $4,329,343. Some of this difference in cost can be associated with a 3 year delay (2009-2012) in start-up of the demonstration project which incurred additional costs (See White Paper 15 May 2013). Regardless, a complete operational program with monitoring effort proportional to what was conducted on the demonstration project would be on the order of $5,476/ha to implement with current technology.

Table 7. Estimated Budget to Aerially Apply DMN Baits with an Internal 80 mg Acetaminophen Tablet over a 500 ha Site Including Monitoring of Brown Tree Snake Numbers, Rodent Trapping and Monitoring, and VHF Tracking for Determination of Bait Fate

Estimated Total Cost of Aerial control of Brown Treesnakes on a 500 ha Site	
Category	**Amount**
Supervisory Salary (1 FTE)	$81,781
GIS/Field Support (0.58 FTE)	$27,264
Technicians (14.2 FTE)	$1,148,863
Safety Officer (0.22 FTE)	$28,413
Administrative (0.35 FTE)	$39,661
Total Salary	$1,325,981
Vehicle/Maintenance/Fuel	$38,048
Rent/Utilities/Other Services	$22,988
Helicopter Aerial Operations Cost	$517,632
Bait Station DNM cost	$3,564
Rodent Trap Baits	$1,188
Aerial Ops DNM Bait and Flagger costs	$180,000
Equipment	$42,731
Supplies	$21,120
Operating Total	$827,271
Total Direct Costs	$2,153,252
Overhead	$584,608
Total	$2,737,860

Estimated Cost of Bait Drops Using an Automated Delivery System.

If an automated delivery system is successfully developed and assumptions on performance parameters are correct the automated system should reduce overall costs by about 13%. This assumes that all monitoring effort and support are similar to the demonstration project. In this scenario the reduction in cost is solely associated with the reduction in cost of aerial operations. The resulting operational cost estimate given these caveats is $2,376,554 in FY15 dollars. The estimate here is an operational estimate and does not include the purchase price of equipment as their currently is no cost estimate. This equipment would be annuitized over a 5-8 year life and presumably operational savings would provide a significant return on investment.

As mentioned in previous section of the Cost and Performance section of this report there may be other sources of savings in an operational brown tree snake control program. Bait station monitoring is the single costliest aspect of an operational control program. Combined with rodent monitoring and transect establishment, monitoring efforts account for more than half of the total costs of the control effort. There are several options with respect to monitoring in support of an operational control program that could provide substantial cost savings.

One option is to reduce the number of transects established for monitoring. As currently designed there would be 490 transects established of which only 54 would be randomly sampled in each bait station or rodent monitoring session. While this number of transects would ensure a drop site spatial coverage of 1 transect per ha, this level of coverage may not be necessary. For example, reducing the number of transects by 56% to 216 (2.3/ha) may provide suitable coverage and still allow a 4:1 ratio of transects to randomly select from for each monitoring session and prevent entrainment of the BTS to bait stations. If this strategy were implemented a commensurate decrease in transect establishment and monitoring costs may be possible, saving almost $650,000 on operational costs per year. Combined with an automated aerial bait delivery system this could bring overall costs down to about $2.1 million for a 500 ha site.

While the demonstration project clearly indicated the aerially delivery of acetaminophen treated DNM bait technology can successfully and significantly reduce brown tree snake numbers, there are still many aspects of the technology that can be further developed and refined. As with many technology transfer efforts, there were many refinements and changes to the technology subsequent to development. Many of the aforementioned developments could lower operational costs substantially. USDA, WS, NWRC and public and private partners are currently working on technologies to help develop these cost savings.

8.0 IMPLEMENTATION ISSUES

8.1 REGULATORY COMPLIANCE

A substantial regulatory burden must be addressed prior to any field operation. All field operations to control Brown Treesnakes would be subject to the federally required NEPA process. Wildlife Services requirements are under Animal and Plant Health Inspection Service (APHIS) NEPA Implementing Procedures at seven CFR Part 372.5(c)(2)(i). This CFR encompasses projects that result in death of a large number of animals or a large proportion of the population, projects which may adversely affect T&E species, and projects with uncertain environmental impacts. The NEPA document would need to be reviewed and supported by the Department of Interior U.S. Fish and Wildlife Service including a Biological Opinion and Section 7 Endangered Species Act consultation. A federal take permit would need to be obtained from the DOI-USFWS and a permit from the Guam Department of Agriculture for application of toxicant baits. In addition any staff involved in toxicant application will need to take and pass the Guam Department of Agriculture, Pesticide Applicators Examination. Because of WS unique position dealing with wildlife damage issues they have their own NEPA staff to address NEPA requirements for large scale control actions.

Currently flag-baits are deployed by hand from a helicopter at 36 baits per ha, the EPA registered application rate (Registration #56228-34) currently held by the WS, NWRC. Any changes to the application rate would need approval through the EPA which can take 2-3 years. The WS, NWRC technology transfer program can aid in this process to obtain approval for changes, potentially reducing the timeframe to approval.

All aerial operations (contractual or in-house) involving WS staff must have a pre operational safety review of pilot and aircrew per WS Aviation Training & Operations Center (ATOC). All aircraft must be maintained in accordance with Federal Aviation Regulation (FAR) part 135 and FAR Part 91 as applicable, to include 100 hour/annual inspections and compliance with the manufacturer's recommendations for Time Between Overhaul (TBO) and Time In Service (TIS). All aircraft and operators covered by this agreement must be certified under the provisions of FAR Part 135, "Operating Requirements: Commuter and On-Demand Operations and Rules Governing Persons on Board Such Aircraft." In addition to Federal Aviation Administration (FAA) requirements all aircraft involving WS staff must conform to requirements of the WS-ATOC safety manual and program.

Other regulatory considerations include Executive Order 13112 (February 3, 1999) – Invasive Species; Brown Tree Snake Control and Eradication Act of 2004 – H.R. 3479, Section 4; Defense Transportation Regulation Part V Chapter 505 Agricultural Cleaning and Inspection Requirements (29 September 2006); Brown Tree Snake Control and Interdiction Plan, Prepared by: Commander U.S. Naval Forces Marianas, Facilities & Environment N45, August 2004; and Andersen Air Force Base 36 Wing Instruction 32-7004 – Brown Treesnake Management, 15 March 2006. These regulations and directives are for the control of BTS but do not specifically call for the development of aerial delivery of treated baits for landscape control of snakes. Modification may be necessary to encompass large scale operational control programs on Guam.

8.2 END USER CONCERNS

Other non-regulatory implementation issues include cost of deployment of toxicant baits and monitoring associated with a large scale operational program. Wildlife Services and NWRC and public and private partners are currently working on technologies to help develop refinements to the technology that could result in significant cost savings such as automated technologies. These ideas are discussed in Section 7 Cost Assessment.

Non-target bait take would be a concern in any application of toxicant baits to control BTS. Addressing the application and monitoring issues above and having an effective monitoring program would be necessary for any operational program. The Demonstration Project indicated that at least on relatively isolated DOD lands non-target bait take rate was very low. However, expansion of programs into different areas and habitats could increase non-target risk. These risks would need to be evaluated as part of the NEPA and permitting process and may require further research to evaluate. Addressing these issues proactively would reduce potential for delay in initiating operational programs.

Cost of deployment of toxicant baits to control BTS is one of the biggest concerns with a large scale operational program. While the demonstration project clearly indicated the aerially delivery by hand of acetaminophen treated DNM bait technology can successfully and significantly reduce brown tree snake numbers, there are still many aspects of the technology that can be further developed and refined. These developments could lower operational costs substantially. One significant consideration is the amount of effort for monitoring that went into the demonstration plan may not be necessary for an operational plan now that the technology has been demonstrated successfully. Monitoring will likely always be required where toxicant will be applied over large areas but monitoring intensity pre unit area may be reduced. These ideas are discussed in Section 7 Cost Assessment. In addition, automated technologies are being developed that may provide further efficiencies and cost reductions. The USDA, WS, NWRC and public and private partners are currently working on technologies to help develop these cost savings.

Application of baits was affected by a number of factors but wind and rain were of particular concern. Applications should be avoided when winds might exceed 15 kilometers per hour or rain events may occur within 48 h post-drop. Using telemetered baits to track bait fate presented challenges in field application. The issue was that snakes were taking the baits but a large number appeared to regurgitate the VHF transmitter prior to mortality. This fact made determination of fate and take rate difficult. Modification to the type of transmitter used, (e.g. implantable, sealed, transmitters) or other methods (testing for acetaminophen residue) may help with this facet of monitoring which is critical for determining bait fate during implementation.

Rodent monitoring will likely be a necessary component of any operational program. An increase in rodent numbers could negate effects of reducing brown tree snake numbers. The preferred Haguruma style traps for Pacific island rodent trapping are no longer made. Due to this situation standard Tomahawk® type traps were modified to a Haguruma type trigger configuration. Monitoring rodent numbers adds additional time and expense to any operational plan. Since non-native invasive rodent would likely be euthanized in an operational program, this must be factored into regulatory requirements such as Federal take permitting and NEPA documentation.

Non-target bait take would be a concern in any application of toxicant baits to control BTSs. Addressing the application and monitoring issues above and having an effective monitoring program would be necessary for any operational program. The Demonstration Project indicated that at least on relatively isolated DOD lands non-target bait take rate was very low. However, expansion of programs into different areas and habitats could increase non-target risk. These risks would need to be evaluated as part of the NEPA and permitting process and may require further research to evaluate. Addressing these issues proactively would reduce potential for delay in initiating operational programs.

8.3 PROCUREMENT ISSUES

There was a single private contract supplier for helicopter services with capability for doing bait drops on Guam. Costs associated with this service were much greater than anticipated (See Section 7 Cost Assessment). Meeting the demands of implementing larger scale programs and dealing with sole source contractual issues and costs may be problematic. Wildlife Services operates their own fixed and rotary wing aircraft and as such have fully trained support staff, pilots, aircrew and policy and procedures to support flight operations. If large scale operations justifying use of WS aircraft are implemented this could facilitate performance of operational programs.

Currently the NWRC is the only manufacturer of the specially formulated acetaminophen tablets for use in aerial bait drops. NWRC could easily meet demand for tablets on the aerial bait drop demonstration project but larger scale operations may require expansion of staff, facilities and equipment to meet demand. While equipment for hand baiting is readily available, equipment being developed for automated delivery of baits is not. All automated equipment is custom built and unique to this application and would likely need to be sole sourced to the supplier. This sole supplier situation is likely to remain the case in this niche market without substantial commercial growth potential. Currently automated delivery and equipment are in a research test phase of development by WS, NWRC with further information forthcoming.

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9.0 REFERENCES

Brooks, J.E., P.J. Savarie, and R.L. Bruggers. 1998a. the toxicity of commercial insecticide aerosol formulations to brown tree snakes. *The Snake* 28:23-27.

Brooks, J.E., P.J. Savarie, and J.J. Johnson. 1998b. The oral and dermal toxicity of selected chemicals to brown treesnakes. *Wildlife Research* 25(4):427-435.

Clark, L., and J. Shivik. 2002. Aerosolized essential oils and individual natural product compounds as brown treesnake repellents. *Pest Management Science* 58:775-783.

Clark, L. 2003. QA-956 Final Report – Efficacy of aerial broadcast baiting in reducing brown treesnake numbers. USDA/National Wildlife Research Center, Fort Collins, CO 11 p.

Clark, L. and P. J. Savarie. 2012. Efficacy of aerial broadcast baiting in reducing brown treesnake numbers. *Human–Wildlife Interactions* 6(2):212–221.

Engeman, R.M., and D.S. Vice. 2001. Objectives and integrated approaches for the control of brown treesnakes. *Integrated Pest Management Reviews* 6:59-76.

Fritts, T. H. 1988. The brown tree snake, *Boiga irregularis*, a threat to Pacific islands. U. S. Fish and Wildlife Service, Biological Report 88 (31). 36 pp.

Fritts, T.H. 1990. Risks to infants on Guam from the bites of the brown treesnake. *American Journal of Tropical Medicine and Hygiene* 42:607-611.

Fritts, T. H., and M. J. McCoid. 1999. The threat to humans from snakebite by snakes of the genus *Boiga* based on data from Guam and other areas. In G. H. Rodda, Y. Sawai, D. Chizar, and H. Tanaka (eds.), Problem snake management: the habu and the brown treesnake, pp. 116-127. Cornell Univ. Press, Ithaca, New York.

Fritts, T. H., M. J. McCoid, and D. M. Gomez. 1999. Dispersal of snakes to extralimital islands: incidents of the brown treesnake *(Boiga irregularis)* dispersing to islands in ships and aircraft. based on data from Guam and other areas. In G. H. Rodda, Y. Sawai, D. Chizar, and H. Tanaka (eds.), Problem snake management: the habu and the brown treesnake, pp. 209-223. Cornell Univ. Press, Ithaca, New York.

Hall, T.C. 1996. Operational control of the brown treesnake on Guam. *Proceedings of the Vertebrate Pest Conference* 17:234-240.

Hayter, A. J. (1989), "Pairwise Comparisons of Generally Correlated Means," Journal of the American Statistical Association, 84, 208–213.

Hocking, R. R. (1985), The Analysis of Linear Models, Belmont, CA: Brooks/Cole.

Perry, G., E.W. Campbell, III, G.H. Rodda, and T.H. Fritts. 1998. Managing island biotas: Brown Treesnake control using barrier technology. Pages 138-143 *in* R.O. Baker and A.C.

Crabb, editors. Proceedings 18th Vertebrate Pest Conference. Univ. of California, Davis, California.

Rodda, G.H., T.H. Fritts, and P.J. Conry. 1992. Origen and population growth of the brown treesnake, *Boiga irregularis,* on Guam. *Pacific Science* 46:46-57.

Rodda, G.H., and T. H. Fritts. 1992. The impact of the introduction of the colubrid snake *Boiga irregularis* on Guam's lizards. *Journal of Herpetology* 26:166-174.

SAS Institute. 2011. SAS/STAT User's Guide V. 9.3. SAS Institute, Cary, NC, USA.

Savarie, P.J., D.L. York, J.C. Hurley, S. Volz, and J.E. Brooks. 2000. Testing the dermal and oral toxicity of selected chemicals to brown treesnakes. *Vertebrate Pest Conference* 19:139-145.

Savarie, P.J., J.A. Shivik, G.C. White, J.C. Hurley, and L. Clark. 2001. Use of acetaminophen for large-scale control of brown treesnakes. *Journal of Wildlife Management* 65(2):356-365.

Savarie, P.J., and K.Tope. 2004. Potential flotation devices for aerial delivery of baits to brown treesnakes. *Vertebrate Pest Conference* 21:27-30.

Savarie, P.J., T.C. Mathies, and K.A. Fagerstone. 2007. Flotation materials for aerial delivery of acetaminophen toxic baits to brown treesnakes. Pages 218-223 *in* G.W. Witmer, W.C. Pitt, and K.A. Fagerstone, editors. Managing vertebrate invasive species: proceedings of an international symposium. USDA/APHIS/National Wildlife Research Center, Fort Collins, Colorado, USA.

Savidge, J.A. 1987. Extinction of an island forest avifauna by an introduced snake. *Ecology* 68:660-668.

Shwiff, S.A., K. Gebhardt, K.N. Kirkpatrick, and S.S. Shwiff. 2010. Potential economic damage from introduction of brown tree snakes, *Boiga irregularis* (Reptilia: Colubridae), to the islands of Hawai`i. *Pacific Science* 64:1-10.

Shivik, J.A., P.J. Savarie, and L. Clark. 2002. Aerial delivery of baits to brown treesnakes. *Wildlife Society Bulletin* 30(4):1062-1067.

SOP FP 034.00. Recovery and handling of animals found dead during routine field activities. 6 May 2010. NWRC Standard Operating Procedure. 2 pp.

SOP LP 004.00. Acetaminophen tablet preparation. 31 October 2001. NWRC Standard Operating Procedure. 6 pp.

Vice, D.S., and D.L. Vice. 2004. Characteristics of brown treesnakes *Boiga irregularis* removed from Guam's transportation network. *Pacific Conservation Biology* 10:216-220.

Wiewel, A. S., A. A. Y. Adams, and G. H. Rodda. 2009. Distribution, density, and biomass of introduced small mammals in the Southern Mariana Islands. Pac. Sci. 63: 205-222.

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