Changes in relative abundance and size distributions of invasive Brown Treesnakes during landscape-scale aerial baiting using novel and standard monitoring methods, and alternative baits for targeting large snakes

BY
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AN ABSTRACT OF THE THESIS of Ella L Norris for the Master of Science in Biology presented December 1st, 2023.

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The Brown Treesnake ***(***BTS; *Boiga irregularis)* is recognized among the world's worst invasive species for its impacts on the native species diversity, ecosystem services, and economy of Guam. An automated aerial delivery system (ADS) is being used at increasing scales for landscape-scale suppression of BTS. We analyzed BTS responses including lure contact rates as relative abundance index and size distributions using data collected over ~2 years using a novel camera-platform monitoring method. We evaluated spatial and temporal effects of ADS treatments in a 55-ha study site surrounded by a snake-proof barrier. We compared this to the data collected in an untreated reference site. Our objectives were to evaluate: 1) short term trends in BTS detection rates at a given location; 2) change in relative BTS encounter rates during a 9-month period when ADS stopped compared to an untreated site; 3) spatial heterogeneity in BTS encounters by considering variation among subplots; 4) immediate BTS responses to multiple ADS re-applications; 5) effects of ADS suppression on BTS size distributions; 6) the accuracy and practicality of camera-platform monitoring compared to that of an established monitoring method also used during this study; and 7) the effectiveness of an alternative bait intended to

target the BTS that persist after standard ADS treatments as a tool to increase probability of mortality among larger BTS size classes.

Our results indicate that: 1) BTS detection rates increased over the first nights of a new camera-platform placement, then leveled off and remained stable after ~5 nights; 2) BTS detection rates were significantly lower in the treated study site but increased following a break in ADS treatment, approaching BTS detection rates similar to that of the untreated site after ~7 months; 3) BTS detection rates showed a delayed but significant negative response to repeated ADS treatments; 4) in both study sites, subplot variation in BTS detection rates was significant among some subplots; however, spatial heterogeneity was no longer significant among the treated subplots after repeated ADS applications; 5) BTS size distribution was skewed toward larger snakes in the treated study site; 6) our camera-platform observations were validated through comparison to data collected during the same study by the established monitoring method, and provided additional data that the established method is incapable of; 7) the bait intended to supplement ADS treatments effectively attracted BTS in the study site after ADS treatments, and outperformed other BTS baits that are less practical for ADS. Our results suggest that the camera-platform monitoring method is effective for monitoring changes in BTS detection rates over short and long-term periods. We show that spatial variation in BTS encounter rates is greatly reduced following ADS treatments, allowing for reduced monitoring effort to account for heterogeneity. This method allows for detection of size-dependent effectiveness of ADS on BTS and size distributions of snakes remaining on the landscape following treatments. These advancements are highly applicable to landscape-scale BTS suppression on Guam, and ultimately improve our capacity to practice wildlife management and restoration at an ecologically meaningful spatial and temporal scale.

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My thesis research consisted of analyzing large datasets produced through the considerable research efforts by USDA-NWRC and their RCUOG contractors. Shane R. Siers was the Principal Investigator for these projects, and created the original study designs and novel wildlife monitoring tools that lead to my thesis. I worked a research associate and a lead technician on the projects prior to beginning my degree, but I could not have completed the work without the hard work of RCUOG research associates Alyssa C. Taitano and Jeried E. Calaor, who contributed immensely to challenging field work and endless game camera data review, and Rachel M. Volsteadt of USDA-NWRC, who curated the large dataset produced. Administrative support during these projects was provided by the Research Corporation of the University of Guam, including Aubrey Moore and Cathleen Moore-Linn. The studies were funded by the U.S. Department of Defense and by the U.S. Department of the Interior Office of Insular Affairs.

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Chapter 1:

Introduction

Changes in relative abundance and size distributions of invasive Brown Treesnakes during landscape-scale aerial baiting using novel and standard monitoring methods, and alternative baits for targeting large snakes

By Ella L Norris

Introduction: Guam is a tropical island in the North Pacific Ocean with a unique endemic biota. Along with hundreds of migratory shorebirds, fourteen terrestrial bird species are native to Guam. Their abundance and distributions decreased dramatically from the 1950s to 1980s and from South to North on the oblong island (Enbring 1984). Early research considered habitat loss, pesticide use, disease, and ultimately concluded that predation by the Brown Treesnake *(Boiga irregularis;* BTS*)* was a probable cause (Savidge 1986, Rodda et al. 1992). Sparse long-term data of Brown Treesnake observations illustrate that the geographic pattern of BTS expansion correlates with the pattern and timeline of bird declines (Jenkins 1983, Wiles et al. 2003).

Early BTS sightings occurred in the early 1950s in Southern Guam and reached the North of the island in the late 1970s. In the 1970s, bird surveys found that bird ranges had shrunken to the North and reported no bird sightings in Southern Guam. In the 1980s, population estimates for most remaining Northern bird species saw sharp declines. Simultaneously, estimated BTS population densities reached up to 100 snakes per hectare, whereas a typical density for snakes of similar size to BTS is 2 snakes per hectare (Rodda and Savidge 2007). In the following decade, BTS densities declined as they overshot their carrying capacity; they have remained relatively stable since the 1990s at 20-50 snakes per hectare depending on the habitat (Fritts and Leasman-Tannter 2001).

Today, the diversity of Guam's forest bird species has plummeted by 70%. Out of the 14 native species only persisted in the wild; others were extinct, extirpated, or persisted only through captive breeding programs. Declines in native lizard abundance and species extirpations occurred as BTS diets shifted following island-wide bird decline (Rodda and Fritts 1992). Loss of ecosystem services like seed dispersal and insectivory has cascading ecological impacts, ultimately depleting Guam's forest health and ecological resilience (Rogers et al 2017, Caves et al. 2013, Rogers et al. 2012, Freedman et al. 2018*)*. The ecological devastation of the BTS invasion on Guam has been recognized as one of the worst ecological invasions worldwide, yet continues to challenge wildlife managers on Guam (Diagne et al. 2021 , Rodda and Savidge 2007).

Wildlife management and research on Guam continues to improve and expand methods for BTS interdiction and suppression, and native species reintroduction. In this study we use a novel BTS monitoring method to evaluate the efficacy suppression, with attention to spatial and temporal variability in its effect to suppress BTS at a landscape-scale. We consider the management applications of the novel monitoring method compared to a traditional one, and of a baiting method that targets BTS that are the most likely to resist current suppression efforts. This research may contribute to ongoing BTS management efforts by increasing our understanding of BTS responses to our suppression efforts and enhancing our ability to quantitatively measure our progress toward wildlife management goals. Landscape-scale monitoring and suppression also could improve our ability to manage BTS across areas targeted and mitigate the likelihood of economic damage, human-wildlife conflict, and accidental transportation from Guam.

BTS management objectives include both damage mitigation and ecosystem restoration. Ongoing BTS management and interdiction efforts are considerable, which is exemplified by

BTS-allocated funding. The Department of the Interior alone contributed $2.8 million for the 2018 fiscal year, $2.9 million for 2019 and $3.4 million for 2020, toward BTS management efforts (Joshua 2018, Joshua 2019, Joshua 2020). Of the $4.1 million in funding for BTS control efforts in the fiscal year of 2021, more than half was allocated to interdiction and control (Joshua 2021). The rest of the 2021 funding was divided amongst various government and federal partners toward research, rapid response coordination, and Guam Power Authority BTS data collection (Joshua 2021). Control methods include visual surveys and manual removal, snake-proof perimeter fences, trapping with live lures and canine detection. These control methods are expensive and labor intensive. They are limited to priority areas, mostly consisting of transportation hubs like airports and cargo areas, with the objective of reducing the possibility of BTS stowing away and being accidentally introduced to another location (Fritts & Scott 1985, Clark et al. 2018).

The allocation of funding and agency efforts reflects a recognized urgency in interdiction, preventing the accidental transportation of BTS to other snake-free Pacific islands. Prevention is the most effective management solution to protect places like Hawai'i, which receives frequent transportation vesicles from Guam and has a similarly vulnerable island ecosystem and could experience a repeat of the negative effects on Guam at an even larger scale. In addition to human-wildlife conflicts such as entering homes and inflicting bites, BTS have interfered with Guam's economy by damaging electrical infrastructure and causing power outages. Over the decades this has accrued to considerable costs in infrastructural repair and loss of economic productivity (Fritts 2002, Fritts et al. 1994, Fritts et al. 1987). Costs of ongoing management efforts and the irreversible loss of ecosystem services are immeasurable. These negative effects of BTS on Guam could be amplified in a larger and more populated state like Hawai'i. Estimates demonstrate that the cost of a BTS introduction to the Hawai'ian islands would far outweigh the cost of interdiction efforts.

Wildlife restoration, such as the reintroduction of native avifauna, remains an ultimate, secondary objective of BTS management on Guam (Brown Tree Snake Technical Working Group 2020). However, this goal has remained elusive due to limitations in reliable and affordable methods to remove BTS from large enough landscapes to support native species recovery. Traditional BTS management methods, such as trapping and visual surveys, are too costly and labor intensive to be practical. A recently developed technology for BTS suppression has made advancements toward BTS interdiction and landscape-scale suppression that improves on cost and efficacy of traditional methods (Siers et al. 2020a, b; Nafus et al. 2022). The Aerial Delivery System (ADS), developed by USDA-APHIS-NWRC and Applied Design Corporation (Boulder, CO), includes a system for automatic assembly of bait cartridges and an aircraft-mounted module that dispenses the cartridges while in flight. The bait cartridges are cardboard tubes containing a 4-6 g dead neonatal mouse (DNM) to which an 80 mg acetaminophen tablet is adhered. Upon ejection from the aircraft, the mouse bait tangles in the treetops where they are accessible to arboreally-foraging Brown Treesnakes. DNM baits are attractive to BTS (Shivik and Clark 1997) and acetaminophen is highly toxic to BTS (Savarie et al. 2000, Siers et al. 2021) but determined to have no concerning unintended impacts on groundwater or non-target species (Johnston et al. 2002). Both automated aerial baiting and manual baiting have been demonstrated to significantly reduce BTS abundance in sites up to 110 ha, the largest scale tested to date (Clark and Savarie 2012, Dorr et al. 2016, Siers et al. 2020b, 2021). A ~40% reduction in relative BTS abundance has been demonstrated after aerial bait application, with reduced activity still evident after 12 months (Siers 2020b). Repeated applications could achieve and maintain a reduced BTS abundance, potentially improving our capacity for interdiction and progressing toward wildlife restoration goals. After a decade of research and development, the system has become an operational tool for use by Wildlife Services to apply baits as contacted by any wildlife manager.

With ADS comes new possibilities for fast and affordable BTS suppression toward both objectives of interdiction and wildlife restoration. Theoretically, there should be some threshold of BTS suppression that could allow reintroduced birds to persist (Pollock et al. 2022, McElderry et al. 2021). Experiments suggest that ADS treatments alone could eventually lead to BTS eradication, but timelines can be shortened by including other tools such as visual searching with manual removal and trapping (Nafus et al 2022). The promise that ADS holds will depend, then, on our ability to quantify its efficacy in reducing the threat which BTS pose on the treated landscape. How much impact did ADS have on the BTS population of interest? Have suppression efforts sufficiently reduced the probability of BTS being transported to other islands? Have we significantly reduced the risk of BTS predation on a native species? Until now, methods for measuring BTS presence in a landscape have yet to match technological advances in ADS methodology.

For preservation of Guam's natural heritage, biodiversity, and ecosystem functions, it will be imperative that we make advancements in the BTS monitoring tools that evaluate control measures and inform important management decisions (Savidge 1987, Atkinson 1996, Rodda and Savidge 2007, Caves et al. 2013, Diagne et al. 2021). Traditional BTS monitoring methods are either imprecise, or are costly and labor intensive, thus fall short in their ability to assess our advancing progress toward important management goals. The de-facto-method for monitoring BTS suppression is "bait tube" monitoring, where 4-6g dead mice are placed in plastic PVC tube bait stations suspended from vegetation. The rate at which baits are removed from these bait tubes is used as an index of relative BTS foraging activity and abundance. Bait tube monitoring data provides information about the relative reduction in BTS foraging activity after ADS, but it does not necessarily tell us how effectively we have reduced the risk of BTS predation on a native species. Further, this method provides no information on the snake taking the bait. Bait tube monitoring targets snakes that take dead mouse baits, similar to the baits dropped by the ADS, providing no information about snakes that may not be willing to take dead prey. Hence, this measurement doesn't provide data required for assessing the BTS population dynamics, or management decisions at a landscape-scale.

In order to effectively apply ADS (or any suppression method) to efficiently achieve wildlife management objectives management goals of interdiction and restoration, we must be able to accurately measure its impact on BTS populations. In pursuit of these developments, several studies have been completed to monitor the efficacy of ADS in reducing BTS activity in a study site. A novel method for measuring suppression effects of BTS, hereafter referred to as camera platform monitoring, has recently been developed by USDA-NWRC for use by Wildlife Services (Siers 2021, Siers et al. in prep). The camera platform apparatus is composed of a commercial wildlife camera mounted face-down above a chamber containing a live mouse lure. Because BTS don't reliably trigger thermal motion sensors, time lapse photographs are recorded with an automatic timer. Recording begins at 18:00H and ends at 06:00H when BTS are most active (Siers et al. 2018). During a trial, a camera platform system records the frequency and duration of BTS attempts to predate the live lure. This allows for assessments of BTS size and tells us how long an individual of a prey species may exist on the landscape until it is encountered by a foraging BTS, which can inform decisions about the survival probability of reintroduced species (McElderry et al. 2022).

When compared to the established method for monitoring relative BTS abundance, camera platform monitoring advances our ability to evaluate the effect of management efforts by improving on the continuity and certainty of bait tube data, with an additional component of BTS size data that bait tube monitoring is not capable of (Siers et al. in prep). While a bait tube collects a single binary data point per sampling episode (bate taken or not taken), a camera platform monitoring collects continuous observations of predation attempts by BTS (count data) throughout each 12h sampling episode. Camera platform monitoring can observe interference by species other than BTS, whereas bait tube monitoring cannot detect when a bait is taken by non-target species unless baits are monitored by cameras. By incorporating size standards in the image, camera platform monitoring can also be used to calculate BTS head measurements, which can then be used to estimate snake body length (Siers 2021). Larger snakes posing greater threat of predation and reproduction; BTS size distributions are important for understanding which size classes are effectively targeted by ADS and which continue to pose a threat on the landscape (Siers et al. 2017a, b; McElderry et al. 2022). Camera platforms can be deployed in the field for longer than bait tubes before requiring maintenance. The setup for both methods requires some vegetative support, but this is more flexible for camera platforms considering that only one camera station needs to be set up rather than a transect of multiple bait tubes. These simple improvements go a long way in fieldwork settings when terrain is rugged and difficult to navigate. The improved efficiency of field use, in addition to the aforementioned advances in data collection, suggest that camera-platform monitoring is highly applicable to current wildlife management objectives, which will likely be carried out across increasing timelines and spatial coverage.

Bait tube monitoring has previously been used to evaluate ADS effects on relative BTS abundance in Northern Guam, making it an ideal metric to validate the data collected by camera-platform monitoring during its early use. I analyze a dataset that was produced from March 2020 to December of 2021 for a study produced by USDA-NWRC in collaboration with RCUOG. Bait tube monitoring and camera platform monitoring were conducted before and after ADS applications in a treated study site, the HMU (Habitat Management Unit), and an adjacent, untreated reference site, the MSA (Munitions Storage Area). These study sites are both comprised of limestone forest that is typical of the area (Anderson Airforce Base, Yigo, Guam). The HMU is a fenced, 55-ha area where studies have occurred since 2016 that used aerial application of acetaminophen baits as method for BTS suppression (Dorr et al. 2016, Siers et al. 2020a). The HMU is bordered by a snake "ex-closure", a fence which is designed to prevent BTS that are outside of the HMU from entering, however allows snakes within the HMU to exit the area. This comprises a BTS population that is closed to immigration but not to emigration, births or deaths. The fence also prevents ungulates from entering the HMU, leading to visible differences in the understory compared to un-fenced areas. Much of the HMU is dense with head-high ferns and other vegetation, whereas the un-fenced MSA is relatively barren with loose, muddy soil in the parts not dominated by rocky, limestone substrate. The MSA did not receive ADS treatments during or prior to this study, while the HMU received intermittent treatments. Both sites were simultaneously monitored for comparison.

During baiting treatments, radio telemetry of tagged snakes in the HMU revealed that larger snakes were more likely to survive aerial applications of cartridges containing small mouse baits (Goetz et al. 2021). Survival of larger snakes could be due to ineffectiveness of very small mice as baits, the standard 80-mg dose of acetaminophen not being 100% effective for larger snakes (Siers et al. 2021), or the fact that larger BTS on Guam tend to spend more time foraging on the forest floor (Rodda and Reed 2007, Siers 2015). Therefore, “alternative ground baits” (AGB) – slightly larger mice that could be integrated into the automated system but fall through to the forest floor, potentially carrying greater doses of acetaminophen – have been proposed as a possible solution (Siers et al. 2019). To test this technique, AGB were deployed along with other larger baits that could be manually dropped from helicopters (rats and small poultry) in the HMU and MSA to evaluate their potential for targeting larger snakes.

The field work for this study was conducted by the USDA-National Wildlife Research Center (Barrigada, Guam), in collaboration with the Research Corporation of the University of Guam. I performed the majority of the field work for these studies as the lead technician for the camera platform monitoring project and the AGB project. I processed most of the data, including reviewing extensive came-camera coverage to obtain BTS count data and head-size estimates from photos. This data processing was done according to a pre-established protocol, to which I made modifications and helped to refine into an improved system that is now the established protocol for camera-platform data processing. The study produced a dataset that spans nearly two years of data collection using camera-platform monitoring to measure BTS responses to aerial baiting treatments. The objective of my thesis was to analyze and interpret dataset to evaluate the efficacy of the suppression efforts employed during this study - including ADS treatments, camera-platform monitoring, and a potential tool to improve BTS mortality during landscape-scale suppression- and finally to report the applications that this research has toward current wildlife management objectives in landscape-scale BTS suppression.

Specifically, I evaluated: 1) short term trends in BTS detection rates at a given location; 2) change in relative BTS abundance during a 9-month period when ADS stopped, using a study site comparison to distinguish between environmental noise and long-term ADS effects; 3) immediate BTS responses to multiple ADS re-applications; 4) spatial heterogeneity in in relation to ADS through the evaluation of variability in detection rates among subplots; 5) effects that ADS suppression has on BTS size distributions; 6) the accuracy and practicality of camera-platform monitoring compared to that of an established monitoring method also used during this study, and; 7) the effectiveness of an alternative bait intended to target the BTS that persist after ADS treatments as a tool to increase probability of mortality among larger BTS size classes during ADS suppression.

The results are summarized in the following chapter under two sub-chapters, which are formatted for separate journal submissions. Their corresponding figures and tables are provided separately in Chapter 3, Appendix A and B.

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Chapter 2: Manuscripts formatted for submission to JWS and WSB

Chapter 2 (A)

Evaluating landscape-scale suppression and monitoring methods: spatial-temporal responses of invasive snake abundance and size-distribution

Prepared for submission to The Journal of Wildlife Management- Research Articles

1 December 2023

Evaluating landscape-scale suppression and monitoring methods: spatial-temporal responses of invasive snake abundance and size-distribution

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ABSTRACT Aerial delivery of toxic baits is used at increasing scales of landscape level suppression of Brown Treesnakes (*Boiga Irregularis*) on Guam. We evaluated the spatial-temporal effects of aerial baiting treatments on the relative abundance and size distributions of Brown Treesnake in a 55-ha, forested study site enclosed by a snake-proof fence. We analyzed data collected using a novel game-camera method over a ~2-year study period. We used reference data from an adjacent, un-treated study area to control for external factors when modeling Brown Treesnake responses to aerial treatments. Our objectives were to evaluate: 1) short term trends in Brown Treesnake detection rates; 2) long-term effects of aerial suppression and the influence of environmental noise through study site comparison; 3) the immediate and compounded effect of repeated aerial suppression treatments; 4) spatial heterogeneity of Brown Treesnake detection in response to aerial treatments; 5) aerial treatment effects on snake size distributions, and; 6) the comparative outcomes of the novel camera platform monitoring method and the priorly established bait tube monitoring method. Our results indicate that: 1) Snake detection rates exhibited a predictable temporal response which are innate to the monitoring method or the study animal's behavior, 2) snake detection rates showed significant responses to treatment, and these responses also varied predictably over time in the absence of aerial suppression treatments while detection rates in the reference site varied unpredictably; 3) repeated treatments have a significant effect on snake detection rates, but the effect may be delayed, insignificant or undetectable after a single treatment application; 4) spatial heterogeneity significantly decreased in response to aerial treatments and was no longer detectable after repeated treatments, and was distinguishable after a single treatment; 5) snake size distribution skewed higher in the treated site but showed unexpected responses following aerial treatments; 6) camera-platform and bait-tube monitoring methods detected similar trends in relative snake abundance over time. We followed the latter observations with a discussion of the comparative effectiveness of both monitoring methods in the context of current wildlife management objectives considerations for either monitoring method.

KEYWORDS adaptive wildlife management, aerial treatments, applied ecology, *boiga irregularis,* Brown Treesnake, camera trap abundance, invasive reptile, island ecosystem, landscape level conservation planning, relative species abundance, risk assessment, modeling spatial-temporal response, reptile body size distribution, suppression and eradication, wildlife monitoring methods.

The invasive Brown Treesnake ***(****Boiga irregularis)* is the subject of extensive wildlife management efforts after decades of negatively impacting native species diversity, ecosystem services and the economy, and causing human-wildlife conflict on the Pacific Island of Guam (Savidge 1986, Rodda et al. 1992). Since the accidental introduction to Guam in the 1950s, the abundance and distribution plummeted from the south to the north of the island in a pattern that correlated with the range expansion of Brown Treesnake (Jenkins 1983, Wiles et al. 2003). Today, Brown Treesnake are recognized as the major cause for Guam's 70% loss in species diversity of terrestrial birds; 10 of 14 species are extinct or extirpated (Savidge, 1987). Declines in native lizard abundance and species extirpations were observed as avian prey became scarce and Brown Treesnake diets shifted toward lizard (Rodda and Fritts 1992). These species losses interrupt ecosystem services like seed dispersal and insectivory and has cascading ecological impacts, ultimately depleting Guam's forest health and ecological resilience (Fritts & Rodda 1998, Caves et al. 2013, Freedman et al. 2018, Rogers et al. 2012, Rogers et al 2017*)*. Economic impacts caused directly by Brown Treesnakes include up to $1.7 billion annually in power-outages alone, caused when Brown Treesnakes climb onto power lines (Rodda and Savidge 2007, Fritts & Savidge 1987, Diagne et al. 2021). Loss of biodiversity and ecosystem functions elicit further, indefinable social and economic impacts. Brown Tree snakes on Guam have been recognized as one of the worst invasive species worldwide, and continues to challenge wildlife managers on Guam (Diagne et al. 2021 , Rodda and Savidge 2007). If BTS were to become established in Hawai'i, predicted economic losses could reach $2.4 billion annually, comprising ecological, social, and infrastructural damages, and management costs comparable to those on Guam (Fritts 2002).

Current Brown Treesnake management objectives include damage mitigation, interdiction or preventing their spread, and ecosystem restoration. Wildlife restoration, such as the reintroduction of native avifauna, remains an ultimate objective (Brown Tree Snake Technical Working Group 2020). Current methods are labor intensive and costly, easily exceeding $4.5 million annually in federal funding (Joshua 2018, Joshua 2019, Hoshua 2020, Joshua 2021). The cost of preventing further invasions is much lower than the cost of further BTS invasions, meaning that interdiction efforts are a cost-effective solution for managers. For this reason, efforts are limited to priority areas to reduce the likelihood of BTS entering these areas and causing damage or being accidentally transported to another location (Fritts & Scott 1985, Clark et al. 2018).

Control methods include visual surveys and manual removal, snake-proof perimeter fences, trapping with live lures and canine detection. These methods have been successful for achieving objectives of interdiction and mitigation, however they are not effective tools for eradication or a level suppression at a significant enough level for native species reintroduction. For this reason, USDA-APHIS-NWRC has developed a system for landscape-level suppression based on aerial pesticide application methods that have been effective for suppression and eradication of other invasive vertebrates, such as rodents. The Aerial Delivery System (ADS) was developed by USDA-APHIS-NWRC and Applied Design Corporation (Boulder, CO) (Siers et al. 2021). It has improved the speed and affordability of landscape scale suppression, with applications in both damage mitigation and ecosystem restoration. During an aerial treatment, baits containing acetaminophen are dispensed from a helicopter over a targeted area (Image 4). Carrion baits of 4-6g, each containing an 80mg tablet of acetaminophen, are rapidly dispersed into tree canopy, where they are available to the arboreal predators. Acetaminophen is highly toxic to Brown Treesnakes but is determined to pose no risk for non-species or for groundwater contamination (Johnston et al. 2002, Savarie et al. 2000, Siers et al. 2021). A ~40% reduction in relative Brown Treesnake abundance has been demonstrated after ADS aerial treatment, with reduced activity still evident after 12 months (Siers et al. 2020b). Predictions show that ADS treatments alone could lead to Brown Treesnake eradication in 17 years in an area surrounded by a snake-proof barrier; timelines could be halved by including other tools such as visual searching with manual removal and trapping (Nafus et al 2022). ADS is now being used experimentally by USDA Wildlife Services at increasing scales on Guam as a part of adaptive wildlife management research efforts (Siers et al. 2021).

Efforts toward eradication are underway but the timeline for its achievement is uncertain. For example, progress toward eradication was recently interrupted when Typhoon Mawar destroyed the snake-proof fence surrounding a study area that had undergone ADS treatments since 2020, potentially allowing the immigration of snakes from unsuppressed areas outside the fence. Two of Guam's remaining endemic forest birds (the Ko'ko' and the Sihek) have persisted through captive breeding programs for roughly 30 years; their reintroduction is time sensitive as their likelihood for survival in the wild decreases with time spent in captivity. For this reason, recent research has sought to identify a threshold of Brown Treesnake suppression that could be attained to allow for successful native species reintroductions even if some BTS remain on the landscape. McElderry et. al (2021) modeled probabilities of bird persistence under simulated levels of BTS predation risk, based on life history characteristics and predation vulnerability for 7 bird species that are candidates for reintroduction. They estimated that for bird populations to persist on a landscape with BTS, the annual probability of a bird being encountered by a foraging snake would need to be no more than 7-20%, or a nightly rate of 0.0002-0.0006 snake encounters per night. It is unknown if this level of threat reduction has yet been achieved, because methods to measure the risk of BTS predation, or the rate of nightly snake contacts, have not been sufficiently used to monitor the effects of BTS suppression. Methods to monitor BTS suppression effects including visual surveys, trapping and bait tube monitoring, produce estimates of relative snake abundance, which are not comparable to estimates for predation probability. Yackel Adams et. al (2019) used game cameras to monitor live lure traps before and after trapping removal efforts and observed nightly detection rates of 0.14 and 0.19 snakes per camera night before and after trapping removal efforts. Similar monitoring has not been applied to landscape level Ads suppression efforts over a longer time scale.

So far, the advancement toward landscape level BTS control that was achieved by ADS has yet to be matched by an accompanying monitoring method to evaluate suppression effects. To effectively apply the tool toward management objectives, it is imperative that we quantify the progress being made toward identifiable management objectives, such as a tolerable threshold of BTS predation threat, which may directly inform subsequent management decisions. We sought to quantify the level of risk reduction that is achieved at a landscape scale during Brown Treesnake suppression activity using a novel monitoring method. We considered spatial and temporal patterns in the effects of aerial treatment on Brown Treesnakes, and we compared the efficacy and practicality of the novel method to an existing one to inform wildlife management usage. We evaluated: 1) short term trends in Brown Treesnake detection rates at a given location; 2) change in relative snake abundance during a 9-month period when ADS stopped, using a study site comparison to distinguish between environmental noise and long-term ADS effects; 3) immediate Brown Treesnake responses to multiple ADS re-applications; 4) spatial heterogeneity in relation to aerial treatments through the evaluation of variability in detection rates among subplots; 5) aerial treatment effects on snake size distributions, and; 6) the outcomes of camera-platform monitoring in comparison an established monitoring method. Finally, we discuss the application of these results to current wildlife management objectives in landscape-scale Brown Treesnake suppression.

STUDY AREA

The island of Guam is the oldest and largest (~540 km^2) of the Marianas Islands. It is situated in the Philippine Sea and is part of Micronesia (13.2 to 13.7EN and 144.6 to 145.0EE). Guam's ecology was characterized by geographical remoteness during its evolutionary history and once consisted of unique endemic biota. Native animals included tropical forest birds, shorebirds, endemic lizards, skinks, and insects. However, native species have largely declined since WWII as invasive and introduced species are increasingly problematic. The island's topography and vegetative cover can be characterized by the substrate type, which is divided from North to South. In the south, volcanic clays support semi-arid highland savannas across rolling mountains, and streams, waterfalls, and some man-made reservoirs persist year-round. In the north, elevated plateaus of limestone forest contain diverse, broad-leaf ever-green tree species. Freshwater seeps down into the porous limestone faster than streams can form, but later re-emerges as freshwater springs from the base of the limestone bluffs that skirt the coast. Residential areas occur throughout the island with most urban areas concentrated in central Guam, with some large areas of relatively undeveloped land in both to the North and South. Guam's year-round temperatures fluctuate little (30-31.5 C by day, and 23.5-25 C by night). Rainfall is abundant (>200 cm/year). Seasons are typically divided into *fanuchånan*, or "rainy season" from July to November, and *fañomnåkan*, or "dry season" from February to May. Humidity is higher in rainy season (averaging over 80% humidity) than in the dry season (averaging 75% humidity). The data we analyzed was collected from June 2020 to July of 2021 in a forested area in Northern Guam (Image 1). The study sites were located at Anderson Airforce Base and are referred to as the HMU (Habitat Management Unit) and the MSA (Munitions Storage Area) (Image 2). The HMU is a 55-ha forested treatment area in Northern Guam where prior studies have been conducted to demonstrate Brown Treesnake suppression via ADS (Image 3), which aerially delivers baits containing acetaminophen as a targeted Brown Treesnake pesticide (Dorr et al. 2016, Siers et al. 2020a). The site is surrounded by a snake enclosure, a specially constructed fence which allows snakes to climb out from the inside, but not to enter it. This comprises a population of snakes that is closed to immigration but not to emigration, births or deaths. The MSA is an adjacent, un-managed forested area that is separated from the HMU by an unpaved road. The HMU is free of wild ungulates and has a understory dense with ferns and native vegetation, whereas the MSA is un-fenced and relatively barren. The HMU received aerial treatments during this study. The MSA received no treatment and was considered a reference study site representative of a Brown Treesnake population that is not affected by aerial suppression. Corresponding observations from both study sites were used to differentiate between environmental stochasticity and the effects of aerial treatments on Brown Treesnake detection rates. Each study site was divided into five subplots.

METHODS

We analyzed data on relative snake abundance and snake size that was collected in the HMU and MSA while aerial baiting treatments occurred in the HMU. Datasets included observations from two methods were used to monitor Brown Treesnake detection rates as a proxy for relative Brown Treesnake abundance: the novel "camera-platform" and the established "bait tube" monitoring methods. Observations encompassed over two years of monitoring in the HMU and MSA, from April 2020 to June of 2021 (Figure 1). Data was provided by USDA-National Wildlife Research Center (Barrigada, Guam).

The camera-platform monitoring method was developed recently by USDA-NWRC for use by Wildlife Services (Siers 2021, Siers et al. in prep) (Image 4). During use, game camera time-lapse photos are recorded for 12 hours each night. Photos can then be assessed to produce count data of nightly snake interactions with a live mouse lure, and snake head size can be measured with the use of size standards printed on the lure platform (Image 5). Head sizes can be used to calculate snake Snout-Vent-Length (SVL). In an adaptive management context, camera platform data provides a measurement of how long an individual prey species may exist on the landscape before being encountered by a foraging Brown Treesnake, which can inform decisions about the survival probability of reintroduced species (McElderry et al. 2022). Snake size estimates are useful to understanding which size classes of snakes persist after aerial treatment; the size class has implication of reproduction rates and prey preferences, both of which are informative to assessing and projecting the remaining risk of Brown Treesnakes.

Prior to this study, the "bait tube monitoring" method was adequately used to assess the efficacy of ADS. Bait tube monitoring data collected during the same study period provided an established metric for Brown Treesnake relative abundance to validate camera-platform monitoring data against. 4-6g dead mice are placed in plastic PVC tube bait stations suspended from vegetation. A transect of bait tubes contains 10 bait tubes which are periodically checked to observe the occurrence of baits taken as a binary response. For either monitoring methods, the rate of brown Treesnake detections may be used as a proxy for their relative abundance in the monitored study area.

Statistical Analysis

Camera-platform monitoring was conducted in two Study Sites (HMU and MSA). Data collection was divided into Trials; Trial Numbers were assigned in sequential order as they occurred and did not always occur simultaneously in both sites. During a Trial, camera-platforms collected data simultaneously in each of five Subplots in a given study site. Camera-platforms were placed at a new location in each Subplot the start of every Trial, then remained in that location for 14 nights of data collection, or “Trial Nights” (Image 6).

We addressed the following objectives: 1) short term trends in nightly Brown Treesnake detection rates at a given location; 2) long-term trends effects of aerial suppression and the influence of environmental noise through study site comparison, during a 9-month period when suppression treatments stopped; 3) the immediate and compounded effect of repeated aerial suppression treatments; 4) spatial heterogeneity in the effects of aerial suppression through subplot data comparison; 5) ADS effects on snake size distributions; and 6) the precision and practicality of a novel monitoring (camera-platform) method compared to an established (bait-tube) monitoring method.

For research objectives 1-4, our outcome variable was nightly snake detection rates measured by camera-platform count data. Camera platform data was modeled as count data, with the number of snake contacts per night as the dependent variable. Preliminary data analyses revealed that count data were zero-inflated and over-dispersed ($\sigma 2 / \mu > 1$), demonstrating that the Poisson distribution would not be appropriate. Therefore, the negative binomial distribution was used to account for overdispersion. Research objectives 5 and 6 evaluated response variables other than camera platform detection rates. For Objective 5 we used mixed-effects model with a negative binomial distribution because size data was over dispersed ($\sigma 2 / \mu > 1$). To address objective 6, we grouped snake detection rates from both monitoring methods as count data for monitoring periods when data from both methods was available and considered the management applications for both methods. Data for snake detection rates was over dispersed ($\sigma 2 / \mu > 1$), so we used a negative binomial distribution during analyses.

In all models, random effects were included to account for non-independence of data, and were selected according to each research objective. When appropriate, we included a random effect for trial number was included to account for pseudo-replication in data which was gathered in one location over multiple trial nights. In some models we included a random effect for Subplot. When study site comparisons were performed, random factors were nested with study site to allow the effect to vary by site (e.g. Site |Trial). Some models evaluated data that was limited to 1-2 month time periods. Through our analysis for Objective 1 determined that Trial Night explained short-term variability in snake detection rates, and that the trend did not vary by study site. We included the continuous and quadratic terms for Trial Night as random effects in subsequent models. Study site was used as a fixed effect in models where study site comparisons were warranted to distinguish the effects of aerial treatment from environmental stochasticity. Other predictor terms were specific to each research objective:

Objective 1: Short Term Trends in Nightly Detection Rates

We sought to evaluate whether there was any non-uniformity in the response variable over the 14-night duration of each trial, which was vital to inform subsequent model designs. We tested the following hypotheses: 1a) Brown Treesnake live-lure contact rates in the primary study area (HMU) will vary significantly in relation to the night (1-14) of a given trial; 1b) Because the HMU has received multiple treatments while the MSA has not been treated, the contact rates response curve will be significantly lower in the HMU; 1c): If there is a temporal trend in nightly detection rates innate to the data collection process, and the response is not an effect of treatment, then the trend will be similar in the treated and untreated study sites (HMU and MSA). We first modeled data from the treated study site, then incorporated data from both study sites to evaluate the potential influences of treatment on the temporal trends detected. We tested the effect of the trial night (Night) as a continuous variable in a negative binomial mixed-effects model, and we compared considered an additional quadratic term (Night^2) for the trial night in a model comparison (Table 1a). We included a random effect for trial number (1|Trial) to account for pseudo-replication in data which was gathered in one location over multiple trial nights: *BTS Detection Rates ~ Study Site * Trial Night + Trial Night^2 + (Site|Trial)*.

Objective 2: Long Term Temporal Trends in Snake Detection Rates

We sought to evaluate temporal trends in snake detection rates observed in relation to treatment status in the HMU. We took advantage of an unplanned delay in aerial treatments that occurred during camera platform monitoring, which provided an opportunity to observe the rate of recovery in snake detection with increasing time in the absence of treatment. We also sought to determine the extent of environmental and temporal stochasticity that was detected in camera platform monitoring by comparing coinciding temporal trends in the untreated study site, the MSA. We considered the amount of time since the date of last treatment (February, 2020) as a continuous predictor variable, also considered a quadratic term for time since treatment. We considered the following hypothesis 2a) Prolonged delays in treatment will result in an increasing trend in Brown Treesnake contact rates as the population recovers in the HMU; and 2b) if observed trends are a result of aerial treatment rather than environmental stochasticity, then overlapping observations from the treated and untreated study sites will significantly vary.

Because we determined that Trial Night explained short-term variability in snake detection rates, we included the continuous and quadratic terms for Trial Night as random effects in this model and thereafter. We did not include "Trial" as a random effect because trial numbers would overlap closely with temporal effects of treatments. We first modeled data within the treated study site to determine if a temporal treatment effect was present (Table 2a): *BTS Detection*

Rates ~ Time Since Treatment BTS ~+ (1|Night) + (1|Night^2). We then incorporated data from both study sites and tested interactive terms for study site and time since treatment (Table 2b): *BTS Detection Rates ~ Study Site + Time Since Treatment + Time Since Treatment^2 + (Study Site * Time Since Treatment) + (1|Night) + (1|Night^2).*

Objective 3: Immediate Effects of Repeated Aerial Treatments

We sought to evaluate the relative difference in brown tree snake responses before and after aerial treatment with reference to the untreated study site (Objective 3A). We also sought to evaluate the immediate and successive responses in relative snake abundance during a series of aerial treatments in the HMU, during which MSA data was not available(Objective3B). We used a Before-After Control-Impact (BACI) study design to model responses to treatment across successive time frames and followed up with pairwise comparisons to generate contrasts of factor levels. We tested the following hypotheses: 3a) If aerial treatment has a significant effect on Brown Treesnake detection rates, then the temporal trend in detection rates in the HMU will be significantly different compared to the temporal trend observed in the MSA *before* and *after* treatment; 3b) Because of the previously demonstrated effect of aerial treatment on nightly Brown Treesnake detection rates, the effects of repeated treatments will overcome the effect of environmental stochasticity, and a significant response will be detectable in the HMU without reference to MSA data.

We first evaluated data from both study sites to compare snake detection rates before and after treatments (Figure 3a). The 'before' treatment period included data from both study sites that was collected several months after the last aerial treatment, at which point snake detection rates were recovering and approached detection rates seen in the untreated study site, however, mean detection rates in the "before" treatment group were still lower in the HMU on average. The amount of data for time periods when both study sites were monitored was limited, but data for both sites was defined within the same two-month period before or after treatment. We did not include “Trial” as a random effect because trial numbers would overlap closely with temporal effects of treatments. We introduced a fixed effect for treatment status, which was assigned to data that was collected before and after treatment (Figure 3a), and we continued to use the random effects used in previous models, and we considered interactive and non-interactive models (Table 3a): *BTS Detection Rates ~ Study Site + Treatment Status + (Study Site * Treatment Status)+ (1|Night) + (1|Night^2).*

We then modeled the response a three-part categorical term for Treatment Status to compare data collected in the treated study site *before, between* and *after* treatments to evaluate the effects of successive treatment applications in the HMU. There was no study site comparison in this approach because MSA data for these time periods was not collected. However, previous study site comparisons of temporal trends in detection rates, including long-term and Before-After treatment comparisons, demonstrated that treatment effects in the HMU were consistently detected through analysis of camera-platform monitoring data and were distinguishable from environmental stochasticity detected in the MSA. Two ADS treatments occurred in the HMU within the span of camera-platform data collections (Figure 1). We subset HMU data into time periods representative of three levels of Treatment Status: before, between and after the two aerial treatment applications. We defined the treatment periods as: 1) March 16- April 20, 2021, 2) May 4- June 20, 2021 and 3) July 1- July 28, 2021 (Figure 3a). We incorporated the treatment period as a categorical fixed effect (Table 3c). We did not include “Trial” as a random effect because of the small number of trials present in this limited subset of data, and because trial numbers would overlap closely with temporal effects of treatments: *BTS Detection Rates ~ Treatment Status + (1|Night) + (1|Night^2).*

Objective 4: Spatial heterogeneity in treatment effects

In the treated study site, HMU, aerial applications occurred according to protocol, and were dispersed uniformly above the study site. However, the forest structure in the study site is not uniform, which may have implications on Brown Treesnake foraging behavior or bait accessibility. The HMU was divided into five subplots (5 ha x 5ha) during camera-platform monitoring efforts (Image 2). We evaluated spatial heterogeneity snake detection rates among subplots in relation to successive treatment applications in the HMU, using Subplot as a categorical fixed effect. We considered the following hypotheses, comparing them against the same null hypotheses: 4a) Because of nonuniformity in forest structure within the treated area (HMU), there will be spatial heterogeneity in Brown Treesnake-lure contact rates that is demonstrated by variance in the Brown Treesnake contact rates observed among subplots; 4b**)** Spatial heterogeneity will significantly vary in relation to aerial treatments. We modeled nightly snake detection rates in relation to subplot and treatment status (1= before, 2=between, and 3= after aerial treatments), and we tested for interactions between the terms (Table 4a). We did not include "Trial" as a random effect because of the small number of trials present in this limited subset of data, and because trial numbers would overlap closely with temporal effects of treatments: *BTS Detection Rates~ Treatment Status + (1|Night) + (1|Night^2).*

Objective 5: ADS effects on snake size distributions

Because Brown Treesnakes prey preferences and foraging behaviors vary among size classes, the effects of aerial baiting may vary in relation to snake sizes. We estimated snake lengths (SVL) based on head size measurements from game camera photos. We tested the following hypotheses: 5a) If snake distribution size distribution is affected by aerial treatment, size distributions will vary between the treated and untreated study site; 5b) If snake distribution size distribution is affected by aerial treatment, size distributions will vary over time in relation to treatment applications. We first modeled snake size in relation to study site (Table 5b): *Size ~ Study Site + (Site|Trial).* We then evaluated temporal trends in snake detection rates; first by comparing the long-term trends in size distribution by study site to distinguish whether treatment effect was visible over several months in the absence of treatment. Trial Night was not included as a random effect because temporal variation in snake detection rates should do not necessarily affect snake sizes; Trial was not included because of the limited trial numbers included in the time-series comparisons. Instead, we used Subplot as a random effect, nested with Study Site (Site | Subplot) to account for potential repeated measures of the same snake over multiple trials in a given subplot. We modeled expected snake snout-vent-length (SVL) in relation to study site and month following aerial treatment in February 2020: *Size ~ Study Site + Month + (Study Site * Month) + (Site|Subplot).* We then evaluated the effect of repeated treatment applications on snake size by modeling size in relation to treatment status (*before, between,* or *after)* during repeated treatments in the HMU (Table 5e): *Size ~ Treatment Status + (1|Subplot)*. Lastly, we evaluated treatment effect by comparing the relationship between detection rates and snake size in both study sites; C) snake detection rates by study site (Table 5f): *Size ~ Study Site + Detection Rates + (Study Site * Detection Rates)+ (Site|Subplot).*

Objective 6: Monitoring method comparison

We sought to compare camera-platform monitoring data to bait tube data collected during the same study period to validate the novel method against an established one. We pooled data from both methods that were collected at the same time in the HMU. Observations occurred during the

~9-month period after treatments stopped in the HMU, during which we detected a significant recovery in snake detection rates in camera-platform data (Table 2a). Bait-take rates typically are modeled as a binomial response; however, we modeled bait take rates as count data rather by summing the number of bait-take occurrences (a binary response 1) per transect in a given subplot during every trial. There were 10 baits available per transect during a trial, meaning that there was limited room for increase in detection rates. However, bait takes never exceeded this number, so the number of baits did not present a limitation in observations and allowed for more congruity in comparison of bait take ratees and camera platform detection rates. Data for snake detection rates was over dispersed ($\sigma 2 / \mu > 1$), so we used a negative binomial distribution during analyses. We included a random effect for time nested within subplot to account for random spatial variation in detection rates in subplots specific to either monitoring method. We considered the following hypotheses: 6a) Because camera-platform monitoring and bait-tube monitoring methods both detect relative brown Treesnake abundance, they will detect similar temporal trends that have previously been demonstrated; 6b) Because camera-platform sampling design has much more refined temporal intervals of data collection compared to bait-tube monitoring, it will detect greater variability in temporal trends of relative snake abundance. We plotted data from both monitoring methods according to the date associated with each (Figure 6a). We included time since treatment as a random effect to account for the expected temporal trends that were previously identified during our analysis of long-temporal trends in the HMU (Table 2a).

We used the R package *lme4* to test hypotheses using a negative binomial generalized linear mixed-effects models, using the R package *lme4*. We compared relative influence of predictor variables and candidate models in an Akaike's Information Criterion (AIC) model selection framework. When within-group comparisons were necessary, we performed pairwise comparisons using the *emmeans* function in R. This function performs a Tukey's test, which compares the means of every combination of categorical variables present in the model formula, while penalizing for multiple comparisons to prevent artificial inflation of effect size. We used the *pairwise* function in R to generate contrasts for all pairwise comparisons. To evaluate the biological significance of model outputs, we back-transformed model predictions to obtain estimated mean nightly contact rates relevant to each research objective. We applied a smoothing effect to visualize temporal trends in observed data and model predictions using locally weighted means (LOESS) through the R function *geom_smooth*. When reporting model results, β was used to report beta values, or standardized correlation coefficients.

RESULTS

In the treated study site (HMU) we observed 2,084 snake encounters over 1,109 camera nights, averaging 2 snakes per night. In the un-treated reference site (MSA) we observed 1,633 Brown Treesnake over 403 camera nights, averaging 4 Snakes per night (Figure 1: Timeline).

Objective 1: Short Term Trends in Nightly Detection Rates

We sought to evaluate whether there was any non-uniformity in the response variable over the 14-night duration of each trial in the HMU (Table 1a). We included a quadratic term for trial night, which improved the model compared to the model without (AIC value decreased from 3,973.4 to 3,954.6). The fitted residuals for both models demonstrate that most of the residual values lie between y= -2 and y=2 when plotted, which is a typical distribution pattern for a negative binomial regression (Figure 1c). The fit of model predictions and observed data was greatly improved with the inclusion of the quadratic term (Figures 1a), so terms for Night and Night^2 were used in subsequent analyses to account for variability within trial periods.

According to the model outputs, both terms for trial night were significant (P(Night) < 0.01, β = .266, SE= .044; P(Night^2) < 0.01, β = -.013, SE=.003). Expected nightly contact rates in the HMU began below 1 snake/night on the first night of trial to ~ 1.75 snakes/night on night 5 (+75%), to 2 snakes/night on night 10 (+12.5% from night 5-10).

We incorporated camera platform data from the untreated study site to evaluate the relationship between treatment effect, and any temporal trend in detection rates that was not a treatment effect (Table 1b). We included a quadratic term for trial night, which was established to be effective in the previous section, and which again improved the model compared to a model without (AIC value decreased from 3,973.4 to 3,954.6). We tested an interactive term (Study Site * Trial Night) (Table 1a). For both models with and without interactive terms, the fitted residuals demonstrated that most of the residual values lie between y= -2 and y=2 when plotted, which is a typical distribution pattern for a negative binomial regression (Figures 1f). The interactive term was not significant, and the interactive model was not used in subsequent analysis (P> .1). Terms for Site, Night, and Night^2 were all significant: We found that expected detection rates were higher in the un-treated study site (P(Site)<0.01, β(MSA) = 1.030, SE= .113); detection rates increased significantly by trial night (P(Night^2< .01, β= .222, SE =.034); and the quadratic term for trial night had a negative effect (P(Night^2< .01, β= -.01, SE =.002), indicating that the a negative curve in the trend of increasing detection rates by trial night. According to model predictions, the overall mean detection was 1.88 snakes/night in the HMU and 4.05 snakes/night in the MSA (+1115.4%). In the HMU, the expected mean nightly contacts were 0.94 on Night 1, 1.96 on Night 7 (+108%), and 2.19 on Night 14 (+10.5%). In the MSA, expected mean nightly contacts were 2.22 on Night 1, 5.09 on Night 7 (+129.28%), and 4.86 on Night 14 (-4.7%) (Table 1c). When plotted, model predictions showed a response curve which flattened after roughly five trial nights, which closely fit the nightly trend in detection rates that was observed across all data (Figure 1c), and fit the observed nightly means specific to each study site (Figure 1d). The fitted residuals for Model 2 demonstrate that most of the residual values lie between y= -2 and y=2 when plotted, which is a typical distribution pattern for a negative binomial regression (Figure 1e). We plotted the linear relationship between predicted and observed values, which reaffirmed that predictions were better fitted for the HMU than the MSA (Figure 1f). During our model selection, we also tested models with and without Night and Night^2 and found that including both terms produced the best fitted model. We tested a cubic term for trial night, and interactive term Trial Night * Study Site and found that neither were significant, so we did not include them in further analyses.

Objective 2: Long Term Temporal Trends in Relative Snake Abundance

We sought to evaluate temporal trends in snake detection rates observed in relation to treatment status in the HMU; we modeled the relationship between snake detection rates and the days = passed since ADS treatment as a continuous and a quadratic term. Both terms were highly significant (P (Date) $< .01$, $\beta = -3.0$, SE=.819; P (Date^2) $< .01$, $\beta = .0001$, SE=.00002). Model predictions fit closely with observed trends when plotted (Figure 2a). When plotted, most fitted residual values lie between y= -2 and y=2 when plotted, which is a typical distribution pattern for a negative binomial regression (Figure 2b). Model predictions and observed data show a steady increase in Brown Treesnake contact rates over several months following the ADS treatment; at the start of each respective month, expected detection rates were roughly 0.8 snakes/night in June, 1 snake/night in July (+25%), 1.2 snakes/night in August (+16.7%), 1.5 snakes/night in September (+25%), 1.8 snakes/night in October (+16.77%), 2.2 snakes/night in November

(+22.2%), and 2.6 snakes/night on November 30 (+15%). Overall, nightly snake detection rates increased by 69% from June 1-Novembr 30 in the HMU.

We included a study site comparison and included interactive terms to allow temporal trends to vary between treated and untreated study sites in relation to aerial treatment (Table 2b), which produced predictions better to observed data compared to a non-interactive model (Figure 2c). According to observed data and model predictions, nightly snake detection rates significantly increased with increasing time since the last aerial treatment in the HMU; in the MSA they decreased; terms for Site, continuous and quadratic terms for Time Since ADS, and the interaction (Site*Date^2) were all highly significant (P(Date) $< .001$, $\beta = -2.0$, SE= .70; P(Site) $< .001$, β(MSA)= 150.0, SE< 43.0); P(Date^2) $< .001$, $\beta = .0001$, SE= .00002); P(Site*Date^2) $< .001$, $\beta < -.008$, SE< .002). When plotted, most fitted residual values lie between y= -2 and y=2 when plotted, which is a typical distribution pattern for a negative binomial regression (Figure 2d). In the HMU, model predictions and observed data showed a steady increase in Brown Treesnake contact rates over several months following the ADS treatment; at the start of each respective month, expected detection rates were roughly 1.15 snakes/night in June, 1 snake/night in July (-13%), 1.1 snakes/night in August (-.04%), 1.3 snakes/night in September (+15.4%), 1.5 snakes/night in October (+15.4%), 2.1 snakes/night in November (+61.5%), and 3.2 snakes/night on November 30 (+34.4%). Overall, nightly snake detection rates increased by 64% from June 1-Novembr 30 in the HMU. In the MSA, observed data showed more variability, but showed a negative trend overall. In the MSA detection rates decreased from roughly 6 to 2 snakes per night between June and October 27 (-67% from June to November); data was not collected in the MSA after October 27. When model predictions were plotted against observed data, there was a stronger positive correlation between predicted and observed data in the HMU compared to the MSA (Figure 2e).

We experimentally applied the model developed for HMU across the entire 2-year study period (Figure 2f). The non-quadratic and quadratic terms for time passed since ADS remained significant ($P < .01$). We plotted the model predictions and applied a smoothing method to allow temporal variation in detection trends using locally weighted averages (loess), which can also be applied to forecast non-linear trends. We found that model predictions demonstrate both a recovery response and the suppression response that align with the application dates of aerial treatment; however, there are considerable data gaps, which introduces great uncertainty in interpretation of these results.

Objective 3: Immediate effects of repeated aerial suppression treatments

We sought to evaluate the relative difference in brown tree snake responses before and after aerial treatment with reference to the untreated study site (Figure 3a). Observed mean snake detection rates in the HMU were 2 (SD=3) before and 2 (SD=2) after ADS (+0%). In the MSA they were 3 (SD=3) before and 5 (SD=4) after ADS (+66.67%) (Figure 3b). We modeled detection rates in relation to study site and treatment (Table 3a), to compared data that represented time periods before and after treatment applications. We included a fixed effect for treatment status (here termed "ADS"), categorized as before or after treatment, and an interactive term for Site*Treatment Status to allow responses to vary in treated and untreated study sites. Study site was highly significant ($P< .01$, β (MSA) =.400, SE (MSA) =.10); Treatment effect was significant ($P< .01$, β (After) = -.300, SE=.10); and the interactive term was highly significant (P(MSA* After ADS)< .01, β =.90, SE=.20). We performed pairwise comparisons based on model predictions (Table 3b). We back transformed the model predictions to the response variable, nightly snake detection rates. In the HMU, expected mean snake detection rates were 2.0 before and 1.55 after treatment (-22.5%). In the MSA, expected mean detection rates were 3.25 before and 5.76 after treatment (+77.23%) (Figure 3c). We applied contrasts to all possible combinations of factor levels and plotted each Treatment-Study Site comparison against their associated significance levels, using Tukey-adjusted p-values (Figure 3d). We found that HMU and MSA contact rates varied significantly varied before treatment ($P<.05$), but this variation was more significant after treatment ($P<.01$) as expected snake detection rates *deceased* in the treated area but *increased* in the untreated area (MSA).

We also sought to evaluate the immediate and successive effects of repeated treatments in the HMU by evaluating snake detection rates during one-month intervals that occurred across three treatment periods: 1) before, 2) between, and 3) after two treatments (Figure 3f). Observed nightly snake detection rates averaged at 1) 2.77 before treatments; 2) 3.04 between two treatments, and 3) after both treatments (Table 3d). We included random effects for trial night and Night^2, which accounted for temporal variability that was not related to treatment effects and reduced short-noise in temporal trends (Figure 3g). We found that detection rates did not significantly vary after in Treatment Period 2, after one ADS application (P(Treatment 2) > 0.1, β =.06, SE =.10); detection rates significantly decreased after two treatment applications, in the following treatment period (P(Treatment 3) < .01, β= -0.50, SE =0.10) (Table 3c). When plotted, residuals illustrate that a majority of the residual values lie between y= -2 and y= 2, indicating a typical distribution pattern for a negative binomial regression (Figure 3h). The expected nightly snake detection rates for each treatment period was 1) 2.52 before treatments; 2) 2.67 after the first treatment (+5.95%), and; 3) 1.56 after both treatments (-41.57%) (Figure 3i,

Table 3i). Pairwise comparisons showed that Treatment 3 detection rates were significantly than both prior treatment ($P < .01$ for both comparisons) (Figure 3j).

Objective 4: Spatial heterogeneity in treatment effects

We evaluated variability in nightly snake detection rates among subplots 1-5 during repeated aerial baiting treatments in the HMU (Table 4a). First, we examined spatial heterogeneity in treatment effects, or variance in the response to treatment among subplots. We modeled detection rates in relation to subplot and treatment period, including an interactive term so that we could test for variability in treatment effects among subplots. We found that the response to treatment varied significantly in only one of five subplots after the first treatment application (P(Subplot 2*Treatment 2)<0.1, $\beta = .880$, SE=.47); and in one of five subplots after the second treatment application (P(Subplot 3*Treatment 3)< .05, $\beta = 1\ .880$, SE= .4). When plotted, most model residuals were between y= -2 and y= 2, indicating a typical distribution pattern for a negative binomial regression (Figure 4a). We then evaluated how overall spatial heterogeneity changed in relation to treatment by comparing levels of spatial variability before, between and after the two treatments using pairwise comparisons (Table 4b, Figure 4b). Spatial heterogeneity was apparent before treatments, but decreased in response to treatments (Figure 4c). For each Treatment Period, detection rates in 5 subplots were compared, resulting in 10 total comparisons. In treatment period 1 there was significant variance in 5 of 10 subplot comparisons ($P<0.01$ for 5 of 10 comparisons); in treatment period 2 there was significant variance in 1 of 10 subplot comparisons ($P<0.05$ for 1 comparison); in treatment period 3 subplot variance was no longer detectable among any subplots ($P>0.05$ for all 10 comparisons). Based on these results, subplot-level variability in detection rates decreased by 80% after one treatment, and by 100% after two treatments. With each treatment, expected nightly detection rates decreased in most but not all subplots; between treatment periods 1 and 3, the expected snake detection rates went from 1.35 to 0.81 in Subplot 4 (-40%), from 1.38 to 0.33 in Subplot 3 (-76%), from 0.86 to 0.69 in Subplot 1 (-19%), from 0.22 to .008 in Subplot 4 (-96%) and from -0.63 to -.01 in Subplot 2 (+98%).

Objective 5: ADS effects on snake size distributions

We selected high-quality game camera photos to calculate the SVL for 1,683 snakes in the HMU and 1,127 snakes in the MSA. According to estimated snake SVL from camera platform data, the overall mean snake SVL was 1,285 mm in the HMU (median SVL was 1,261mm) and1,165 mm in the MSA (median SVL was 1,170 mm) (Figure 5a). Our model showed that estimated SVL varied significantly by Study Site ($P<.001$, $\beta(MSA) = -.086$, $SE= .009$) (Table 5a).

We evaluated temporal trends in snake detection rates and found that in the treated study site (HMU) expected SVL increased significantly with each passing months since treatment, except for from August to September ($P(August)>.05$). In the MSA, SVL did not vary significantly from month to month, except for September to October (Figure 5b). In the HMU, expected SVL was approximately 1,190mm in June, and 1,340mm in October (+12.6%). In the MSA, expected SVL was approximately 1,130mm in June and 1,180 mm in October (+4.4%) (Figure 5b). Model residuals fell between y=2 and y=-2 when plotted, demonstrating a reasonable distribution for a negative binomial model (Figure 5c).

We then evaluated the relationship between snake size and repeated aerial treatments in the HMU. The observed mean SVL by Treatment Period was 1) 1,312mm before treatments; 2) 1,243 mm between treatments, and; 3) 1,268mm after the two treatments, resulting in a 5.29% decrease after one treatment and a 3.4% decrease overall (Table 5c). We found that SVL variance decreased significant from Treatment Period 1 to 2 ($P<.001$, $\beta(2) = -.055$, $SE= .009$) and from Treatment Period 1 to 3 ($P< .05$, $\beta(3) = -.03$, $SE= .014$) (Table 5d). According to these model outcomes, the expected SVL for each Treatment Period was approximately 1) 1,323mm before treatments; 2) 1,255 mm between treatments (-5.1%), and 3) 1,275mm after the two aerial treatments (+1.59%), predicting a -3.6% change in SVL overall (Figure 5d). Model residuals fell between y=2 and y=-2 when plotted, demonstrating a reasonable distribution for a negative binomial model (Figure 5e).

We modeled snake snout-vent-length (SVL) in relation to corresponding snake detection rates for both study sites. We found that snake detection rates both had significant effects on SVL, but the effect size was small (<1%) given the large sample size.

Objective 6: Monitoring method comparison

We sought to compare camera-platform monitoring data to bait tube data collected during the same study period to validate the novel method against an established one. When we plotted observed bait take rates and camera platform detection rates as count data, we found that detection rates for both methods demonstrated similar temporal trends (Figure 6a). We modeled long term trends in all detection rates in relation to monitoring method, with time passed since treatment as a random effect (Table 2a). We found that detection rates varied significantly by monitoring method was highly significant ($P<.01$, β(Camera Platform) = - .60, SE= .22). Expected Bait Tube detection rates went from roughly 1.7 in July to 2.6 in December (+ 52%). Expected Camera Platform detection rates went from roughly 1.05 in July to 1.75 in December (+ 66%). When plotted, we found that the predicted snake detection rates followed observed trends closely, with a slightly better fit between predictions and observed data for bait tube data (Figure 6b).

We then compared monitoring method data across shorter, one-month time intervals, which demonstrated higher degrees of temporal variation when plotted compared previous smoothing of long-term trends (Figure 6c). The temporal variability in observed data again appeared similar, even as mean monthly detection for camera-platform data remained lower than bait take during most months (Table 6b). According to model outcomes, detection rates did not vary significantly between the two monitoring methods (P(Method) > .05, Chisq= .79, df=1); detection rates did vary significantly by month (P (Month Since Treatment)<.001, Chisq=130.94, df=8); and monthly variability differed between methods (P(Method * Month)< .001, Chisq=32.45, df=8) (Table 6c). Contrasts showed that the difference in monthly mean detection rates was insignificant for 8 of 9 months snake detection rates for both methods varied similarly monthly, except for one month (P(April<.05) (Figure 6d). Although monthly variability was similar by study sites, camera platform data showed higher sensitivity to detecting variability. There were significant differences in detection rates across one-month increments of camera platform data (P< .01 for all months except September and October), but less significant variation across one-month increments in bait tube data (P > .05 for all months) (Figure 6e, Table 6d). Expected Bait Tube detection rates went from roughly 1.2 in April to 2.4 in November (+100%). Expected Camera Platform detection rates went from roughly .45 in July to 2.9 in November (+ 544%) (Figure 6f).

We found a greater ranges of variance in camera-platform monitoring data overall. Overall, the bait tube detection ranged from a low of 0.812 in July to a high of 3.0 in September (+269.46%); camera platform monitoring ranged from a low of .272 in April to a high of 3.655 in December (+1,243.75%). We compared variability among all monthly detection rates for each method and found that for camera platform data, 9 of 36 month-to-month comparisons were statistically similar (P > .05), 5 of 36 were significant (P<.05), and 22 of 36 were highly significant (P<.001) (Figure 6g).

DISCUSSION

Our results indicated that aerial treatments via ADS had significant effects on relative snake abundance and size distributions in a treated study area, and that camera platform data consistently identified spatial and temporal trends of interest while distinguishing between treatment effects from environmental and temporal stochasticity that was demonstrated in the untreated reference site during the same monitoring periods. We present our discussions in reference to the following study outcomes: 1) Snake detection rates exhibited a predictable temporal response which are innate to the monitoring method or the study animal’s behavior, 2) snake detection rates showed significant responses to treatment, and these responses also varied predictably over time in the absence of aerial suppression treatments while detection rates in the reference site varied unpredictably; 3) repeated treatments have a significant effect on snake detection rates, but the effect may be delayed, insignificant or undetectable after a single treatment application; 4) spatial heterogeneity significantly decreased in response to aerial treatments and was no longer detectable after repeated treatments, and was distinguishable after a single treatment; 5) snake size distribution skewed higher in the treated site but showed unexpected responses following aerial treatments; 6) camera-platform and bait-tube monitoring methods detected similar trends in relative snake abundance over time. The last objective is followed by a qualitative comparison of the practicality of either method in the context of current wildlife management objectives considerations for either monitoring method.

Short term trends in nightly detection rates:

Our results indicated that nightly camera-platform detection rates were *not* uniform across all 14 nights of monitoring, but rather varied consistently in both treated and untreated study sites. During a controlled ecological study, the response of a target species with respect to the monitoring method may not be immediate or uniform. In some cases, organisms may exhibit atypical behavior in response to the alterations in their environment that are entailed by the experiment, but eventually become accustomed to the anomaly and return to normal behavior. Our findings indicate that the observed nightly trend is not a result of the treatment and more likely an effect of the methodology or biological behavior of the study species.

The acknowledgement of a predictable response curve has important implications for how nightly variance can be accounted for. In future camera-platform monitoring studies, a response curve in nightly contacts within a given trial should be anticipated and accounted for. Monitoring periods should always be over 5 nights in length to allow for initial temporal variation, after which contact rates are expected to be uniform for, at a minimum, the remainder of a 14-night trial. One way to account for the response curve is to omit the first few nights of trial data. Or, to increase the data yields per unit of sampling effort, the response curve can be accounted for in model design such as our own by assessing the 14-night response curve, as opposed to assessing single nightly averages that are selected from specific nights within a trial (Table 1c).

Long term temporal trends in relative snake abundance:

Although the delay in ADS treatment was the result of an unanticipated interruption in management plans at the advent of to the COVID-19 pandemic, it provided an insightful opportunity to observe the recovery of relative Brown Treesnake abundance during a lag in suppression activity. This is invaluable information for long-term planning for suppression efforts. It is necessary to consider the possibility of unanticipated alterations to planned management activity, and to use this information in the preparation of a response plan for scenarios where certain management activities, such as aerial suppression, become inaccessible.

During the cessation of treatment in the HMU, we observed a clear pattern of recovery in relative snake abundance. By comparing HMU trends to those observed in the un-treated study site, we can infer with more certainty the expected effects of treatment. Further, we can better understand the degree of unpredictable variability may offset the efficacy of aerial treatments. The study site comparison played an essential role in assessment of both treatment effects and the reliability in camera platform monitoring. The variance in detection rates in the MSA were consistent and stable over time, which suggests that these were not temperamental responses or uncertainty in the monitoring method itself. According to anecdotal observations commonly made by field biologists on Guam, Brown Treesnake activity significantly increases while it is raining. It is possible that effects of seasonality influenced the decline in detection rates observed in the MSA and imply that concurrent trend of increase in the HMU maintained momentum in spite of external pressures. There are too many uncontrolled variables presented by the dataset of consideration, however it is possible that further long-term ecological monitoring with the use of the camera platform method could help to clarify seasonal patterns and begin to untangle the multitude of unexplained factors at play. Until then, we have demonstrated that unpredictable environmental stochasticity can be accounted for in models with sufficient study site comparisons.

Immediate effects of successive aerial treatments:

We compared treatment effects through study site comparison of "before" and "after" treatment effects. Ideally, we would have evaluated "before treatment" data corresponding to a time when HMU detection rates had recovered to match those of the un-treated reference site, however corresponding MSA data was not sufficiently available. Instead, the "before" monitoring period began 2-3 months after the February 2020 ADS treatment, at a time when detection rates were significantly lower in the HMU than in the MSA. This explains why there was significant variance in the study site detection rates before treatments, which were also present after treatment. However, detection rates did not significantly decrease in the MSA after treatment, and they significantly increased in the HMU. Because the MSA and HMU study sites are adjacent and similar in terms of habitat, we consider the MSA to be indicative of Brown Treesnake responses to temporal or environmental stochasticity that can occur in the absence of treatment effects. Thus, without the effect of ADS treatment, we would expect Brown Treesnake counts to significantly increase in the HMU as well as in the MSA, as they were subjected to grossly identical external environmental conditions.

The opposing responses detected at either study site after ADS treatment suggests that the temporal trend in Brown Treesnake activity in the HMU was suppressed relative to the expected trend which was established by the un-treated reference site. This observation applies that treatment did affect snakes in the HMU by effectively preventing the significant increase in Brown Treesnake activity that we observed in the reference site.

There were several months without data collection before the “after” period occurred, which introduced greater possibility of un-treatment related influences on the observed responses. Because the MSA was considered as an untreated reference site, we can infer that the increased snake encounter rate between time periods was not an effect of ADS treatment. Rather, it occurred because of uncontrolled environmental factors that cannot be explained within the parameters of this study.

Treatment effects on spatial heterogeneity in snake detection rates:

We found that snake detection rates observed simultaneously at five different sampling locations in the HMU varied significantly from each other before treatment. The significant variability among subplot reduced substantially after one ADS treatment application and were no longer detectable after the second application. It is worth noting that the reduction in spatial heterogeneity among subplots was detectable before the overall reduction in detection rates could be detected for the overall study site. Comparing patterns in spatial variability may be another useful factor to include when evaluating temporal responses in relative snake abundance.

The difference in Brown Treesnake contacts among subplots could be explained by differences in vegetation structure that may influence Brown Treesnake foraging behavior , or to the proximity of the subplots to varying habitats beyond the study area perimeter. For example, subplots that are bordered by densely forested habitats outside of the HMU may have an influx of prey species, which in turn may increase the likelihood of foraging Brown Treesnake to be observed in the area. Spatial heterogeneity is not specific to the treated landscape where we observed the subplot-level variation in detection rates. Our results do suggest that uniformity in detection rates increases with successive treatments. This is useful to consider in future sampling designs; un-managed landscapes may warrant more intensive spatial coverage to achieve accurate representation of detection rates across the landscape. On the other hand, the decrease in spatial variability decreases as uncertainty in observations increases during times of decreased relative snake abundance. If there is such a relationship between subplot heterogeneity and overall detection rates, we could make inferences about the thresholds for reliable camera platform estimates. It would be extremely useful to define limitations in the management scenarios which camera platform monitoring can be applied to. This would not only prevent ineffective sampling efforts, but it would also identify the areas for improvement that could be prioritized to improve accuracy in monitoring practice and increase the applicability of the technology.

Snake size distribution in relation to aerial treatments:

As the effects of aerial treatment diminished and relative abundance increased in the HMU, the estimated snake size also increased, implying that treatment effects had initially resulted in a smaller size distribution in the HMU. In the MSA, estimated snake SVL remained relatively similar. The correlation between timing of treatment and changes in snake size distribution implies that the large size distribution in the HMU was not a direct effect of treatment. Rather, it was a result of the cessation of treatment.

The positive linear relationship between snake abundance and mean SVL in the HMU suggested that the proportion of large snakes steadily increased over time in the absence of ADS. Conversely, our analysis of repeated aerial treatments suggested that mean snake size distribution skewed smaller immediately after treatments. If we consider camera platform detection rates as a measure of predation threat, we are led to the conclusion that small snakes persisted after suppression activities and were the most likely to pose a threat to prey species following treatment. This contradicts our expectation of large snakes remaining after aerial treatments.

It is possible that snake foraging behaviors changed as inter-species competition was reduced after treatment, which increased the likelihood of small snake interactions with the lure and, we introduced noise to size estimates. Alternatively, it is possible that the camera-platform size estimates picked up on actual fluctuation in the proportion of small snakes in the ADS, which imply that. The positive relationship between snake size distribution and detection rates suggests that when snakes have been effectively reduced by treatment in the HMU, snakes continue to interact with live lures. Further research in this regard could clarify whether this is an indicator of biological effect or a relationship inherent to the observation methodology.

Regardless of the causal relationship, the observed patterns of negative SVL response to treatment suggests that the reason for the larger size distribution of snakes in the HMU may not be fully understood, and that the persisting level of Brown Treesnakes threat that can be detected on a landscape after treatment should not solely be attributed to the persistence of large snakes that evaded death by toxic bait. This has important implications on the future directions of applied research in Brown Treesnake management. So far, most efforts have focused on supplementing aerial bait applications (ADS) with baits that target larger, ground-foraging snakes.

It remains possible that the pattern detected was prone to bias inherent to the monitoring method, which is still in the early stages of its uses in wildlife management. The observed relationship in abundance and snake size distribution could be clarified by comparing camera platform size data to corresponding data obtained through other established methods such as visual surveys and snake capture to manually obtain SVL measurements. Unfortunately, such data has was not available for the study period we evaluated. So far, other metrics for snake size estimates and density estimates have yet to be calibrated with camera platform observations.

Monitoring method comparison:

We compared camera platform data to bait tube monitoring, which has previously been used to monitor effects of aerial suppression treatments via ADS on Guam. We also considered practical applications and utility of both methods as tools for wildlife management going forward. Both methods provide comparable measures relative Brown Treesnake abundance. Through comparisons of corresponding time series data, we found that both methods detected similar long-term trends of recovery in relative snake abundance when treatments were stopped. When we compared mean detection rates at monthly intervals, the relationship was less direct. Bait tube data showed a general increase in detection rates over the first several months, and then a decrease in detection rates over the second half of the monitoring period. The negative trend in bait tube data corresponded to the one simultaneously observed through camera platform monitoring in the MSA. Unfortunately bait tube data was not collected in the MSA during this period, so we cannot determine whether bait tube trends in the HMU could be distinguished from environmental stochasticity in the MSA.

We compared variability among monthly detection rates of both methods and found that camera platform monitoring detected a greater degree of increase in detection rates over the 9-month monitoring period. This could imply that there was more noise in camera platform data; conversely, it could imply that camera platform monitoring returns a more sensitive estimate of biological responses. These findings are not an exhaustive comparison bait tube monitoring has been used extensively in prior studies, and the subset of bait tube data that we measured was relatively small. We also took an unconventional approach to comparing temporal trends detected by each monitoring method; bait tube data is typically evaluated as binomial response data, where we modeled it as count data in a negative binomial model.

We now present a more general comparison of monitoring methods by discussing the distinguishing characteristics of either method. We evaluated the practicality and usefulness of both monitoring methods in the context of current management objectives. We found that the novel method, camera-platform monitoring, offers several important advancements. A bait tube collects a single binary data point per sampling episode (bate taken or not taken). There is inherent uncertainty with the possibility of the bait being taken by a non-target species rather than a snake. Camera platform monitoring collects continuous game-camera data observations of predation attempts by Brown Treesnake (count data) throughout each night of sampling, which may improve sensitivity to the response variable. Camera-platform monitoring has 100% certainty in distinguishing Brown Treesnake detections from non-target interactions, and furthermore provides some feedback on species interactions of concern to management goals. The novel method is highly applicable to adaptive management strategies toward landscape scale ecosystem restoration through native species reintroductions. Camera images can be used to estimate snake size (Siers 2021); size distribution data provides useful feedback about which size classes pose a threat on the landscape (Siers et al. 2017a, b; Nafus et al. 2022). The continuous game camera data records the frequency and duration of Brown Treesnake attempts to predate the live lure. This provides a measure of survival probability, or how long a prey species is likely to go without being encountered by a foraging Brown Treesnake. This can be used for risk assessment, to establish a baseline level of predation risk acceptable for native species reintroduction and determine when that baseline has sufficiently reduced (McElderry et al. 2021, McElderry et al. 2022, Pollock et al. 2022).

Brown Treesnakes demonstrate dietary preferences which influence their likelihood to consume an ADS-distributed toxic bait. The live lure detection rates are independent from the treatment method, which uses carrion baits. The standard carrion baits that are used in bait tubes are similar to the standard ADS baits. This has implications on the ability for this method to detect biological responses among the snakes that are unlikely to ingest the ADS baits, as they are also unlikely to pursue bait tube baits thus may not be represented in bait tube estimates of relative snake abundance. Brown Treesnake that are also targeted by the similar baits used during ADS. This becomes important as those Brown Treesnake are removed from the landscape, which may lead bait take rates to show an artificial decrease in relative Brown Treesnake abundance.

We conclude that Camera-platform monitoring provides multifaceted improvements to management methods for monitoring the biological response of Brown Treesnakes to suppression efforts. The method returns useful data on relative abundance and sizes of Brown Treesnakes. It accounts for the dynamic relationships of spatial and temporal variability as it does so, and it demonstrates an ability to distinguish treatment effects from other variables both during long term trends, and in more immediate responses through nightly observations. This method could have useful applications in other wildlife management scenarios, such other monitoring arboreal or nocturnal species that are difficult to produce abundance estimates of. Currently, its strengths lie in its applications to the field of Brown Treesnake suppression which it was designed for. As the scale of ADS suppression efforts increased on Guam, camera platform monitoring is a highly practical tool for quantifying progress toward wildlife management and restoration goals.

MANAGEMENT IMPLICATIONS

Our results supported the following hypotheses: 1a) Brown Treesnake live-lure contact rates in the primary study area (HMU) vary significantly in relation to the night (1-14) of a given trial; 1b) Because the HMU has received multiple treatments (aerial toxic baiting for Brown Treesnake suppression), the response will be significantly lower in the HMU; 1c): If there is a temporal trend in nightly detection rates innate to the data collection process, and the response is not an effect of treatment, then the trend will be similar in the treated and untreated study sites (HMU and MSA); 2) Prolonged delays in treatment will result in an increasing trend in Brown Treesnake contact rates as the population recovers; *3a)* If aerial treatment has a significant effect on Brown Treesnake detection rates, then the temporal trend in detection rates in the HMU will be significantly different compared to the temporal trend observed in the MSA *before* and *after* treatments; 3b) Because of the previously demonstrated effect of aerial treatment on nightly Brown Treesnake detection rates, the effects of repeated treatments will overcome the effect of environmental stochasticity, and a significant response will be detectable in the HMU without reference to MSA data; 4a) Because of nonuniformity in forest structure within the treated area (HMU), there will be spatial heterogeneity in Brown Treesnake-lure contact rates that is demonstrated by variance in the Brown Treesnake contact rates observed among subplots; 4b) Spatial heterogeneity will significantly vary in relation to ADS treatments; 5a) If snake distribution size distribution is affected by aerial treatment, size distributions will vary between the treated and untreated study site; 5b) If snake distribution size distribution is effected by aerial treatment, size distributions will vary over time in relation to treatment applications.6a) Because camera-platform monitoring and bait-tube monitoring methods both detect relative brown Treesnake abundance, they will detect similar temporal trends that have previously been demonstrated; 6b) Because camera-platform sampling design has much more refined temporal intervals of data collection compared to bait-tube monitoring, it will detect greater variability in temporal trends of relative snake abundance.

Through the data that was available, we established that camera platform monitoring reliably detects spatial and temporal trends, over nightly time scales and across monthly time scales, in relative snake abundance as well as snake size distributions. These findings were affirmed by multiple metrics including study site comparison, evaluation of temporal trends in relation to treatment applications, and through reference to a novel and an established monitoring method. Our observations provide important insight on the practical utilization of the method. We determined that short-term variance in detection rates by trial night must be considered in all future uses of camera platform monitoring data, regardless of treatment or lack thereof on the landscape of interest. We found the study site comparison to be extremely insightful to understanding treatment effects that otherwise may be indistinguishable from unrelated temporal and environmental stochasticity in snake detection rates, and we advise that simultaneous study site monitoring be incorporated in the design of future controlled studies. We found that spatial heterogeneity in detection rates decreased after aerial treatments, however we cannot be certain as to whether this was representative of increased uniformity in snake detection rates, or rather a decrease in sensitivity to snake activity that is inherent to the monitoring method as snake abundance was lowered following treatment. This requires further investigation, as does the relationship between snake size distribution and aerial treatment. Through repeated measures, we observed that treatment led to a decrease in mean snake length, but we also observed overall averages of snake size distribution by study site skewed higher in the HMU. If the rates of snake predation attempt on live lures can be considered an index of the predation threat posed to prey species by brown Treesnakes, then our findings suggest that ADS aerial treatments lead to greater risk posed by smaller Treesnakes, contrary to our expectations. If this finding can be reaffirmed, then it implies future management research should prioritize methods for suppressing and detecting small snakes that continue to threaten prey species after larger snakes have been removed from the landscape.

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Chapter 2 (B)

Evaluation of an Alternative Bait Tool to Increase Target Species Mortality During Landscape-Scale Suppression of the Invasive Brown Treesnake

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Evaluation of an Alternative Bait Tool to Increase Target Species Mortality During Landscape-Scale Suppression of the Invasive Brown Treesnake

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ABSTRACT The Brown Treesnake (BTS; *Boiga irregularis)* is recognized among the world's worst invasive species for its impact on the native species diversity, ecosystem services and economy of Guam. The Aerial Delivery System ("ADS") is a method for aerial application of a toxicant-treated carrion bait that is demonstrated to significantly reduce and maintain the reduction of BTS abundance on landscape scales. During baiting treatments, radio telemetry of tagged snakes in the HMU revealed that larger snakes were more likely to survive aerial applications of cartridges containing small mouse baits. To increase the effectiveness of ADS across a larger range of snake size classes, it has been proposed that ADS treatments could be supplemented with an alternative bait designed to target the BTS most likely to be missed by the typical ADS bait. The ideal supplemental bait would be integrated into the ADS system to enable application across large areas and remote landscapes alongside standard ADS baits. This would minimize any added costs of labor and technology associated with the additional implementation as a control tool. We tested the effectiveness of three large, ground-deployed bait types that could increase probability of mortality among larger snake size classes during Brown Treesnake suppression. We analyzed game-camera images to evaluate the probabilities of bait consumption by Brown Treesnakes and by non-target species in an area undergoing ADS treatments, and we compared observations to corresponding data from an untreated reference area to evaluate variation in snake bait preference in relation to treatments. The carrion baits included a mouse (13-17g), a rat (85-165g), and a chick (85-165g); the mouse bait is larger than the typical (4-6g) "fuzzy" mouse bait used in ADS, but small enough to be incorporated into the ADS with minor modifications. Our results suggest that the baits demonstrate equal likelihood of probabilities of success at luring ground-foraging brown Treesnakes after aerial treatment (Tukey-adjusted p-values $P > 0.1$ in pairwise comparisons), but all baits were widely susceptible to non-target interference. In an un-treated study site there was more variability in probability of success by bait type, suggesting that wildlife managers should consider the local conditions and history of treatment on the landscape when implementing ground baits as a control tool.

KEYWORDS *Boiga irregularis,* Brown Treesnake, foraging ecology, adaptive management, aerial treatments, applied ecology, island invasive reptile, landscape level restoration, conservation planning, risk assessment, suppression and eradication, wildlife methods.

The invasive Brown Treesnake (*Boiga irregularis)* has caused significant ecological and economic damage over decades since its accidental introduction to the Pacific Island of Guam (Savidge 1986, Rodda et al. 1992). Their impacts have included significant reduction in native biodiversity with the extirpation and extinctions of native vertebrates such as lizards and avifauna (Savidge1987, Rodda and Fritts 1992), economic impacts including up to $1.7 billion annually in power-outages alone (Rodda and Savidge 2007, Fritts & Savidge 1987, Diagne et al. 2021), and losses in ecosystem functions Fritts & Rodda 1998, Caves et al. 2013, Freedman et al. 2018, Rogers et al. 2012, Rogers et al 2017). Wildlife management research has been developed and implemented over the past several decades to suppress Brown Treesnake populations, with the goal of reducing their abundance to allow for reintroduction of native species the persist through captive breeding efforts (Fritts & Scott 1985, Clark et al. 2018, Brown Tree Snake Technical Working Group 2020). The Aerial Delivery System, typically referred to as "ADS", is a method for aerial delivery of a carrion bait containing a toxicant lethal to Brown Treesnakes but unimpactful to non-target species (Johnston et al. 2002, Savarie et al. 2000). ADS was developed by USDA-APHIS-NWRC and Applied Design Corporation (Boulder, CO) (Siers et al. 2021). Treatments have been demonstrated to suppress Brown Treesnakes by ~40% across a 110-ha landscape, and reduced activity was maintained for at least 12 months (Siers et al. 2020b). ADS is now being used experimentally by USDA Wildlife Services at increasing scales on Guam as a part of adaptive wildlife management research efforts (Siers et al. 2021).

ADS rapidly deploys carrion lures over a targeted landscape, designed to entangle in the tree canopy where they may be encountered by arboreally foraging snakes. Because brown Treesnake foraging habits and dietary preferences vary ontologically, the effects of ADS treatments on Brown Treesnake are expected to vary ontologically as well (Siers et al. 2017). During baiting treatments, radio telemetry of tagged snakes in the HMU revealed that larger snakes were more likely to survive aerial applications of cartridges containing small mouse baits (Goetz et al. 2021). Survival of larger snakes could be due to ineffectiveness of very small mice as baits, the standard 80-mg dose of acetaminophen not being 100% effective for larger snakes (Siers et al. 2019), or the fact that larger BTS on Guam tend to spend more time foraging on the forest floor (Rodda and Reed 2007, Siers 2015). Large BTS are more likely to forage on the ground, which would reduce their probability of encountering an ADS-bait in the canopy. Further, a single dose of toxicant in a given AGB bait may not induce mortality in the large outlier snakes (Siers et al. 2021). Large snakes are of greater concern to suppression efforts because they contribute the most to reproduction and population growth. Baits that are delivered to the ground, with higher doses of toxicant, would solve these issues.

It has been proposed that a bait that is larger than the typical bait used during ADS could be integrated into the automated system in such a way that they fall through to the forest floor, where their size and placement increase the probability of being consumed by a large, ground-foraging snake (Siers et al. 2019, Siers et al. 2021). Such an alternative bait could contain greater doses of acetaminophen to increase probability of mortality among larger BTS size classes during ADS suppression. A larger size of bait could reduce the likelihood of consumption by potential small, ground foraging BTS, thus increasing the availability of the bait to large BTS (Siers 2017).

The ideal design for a supplemental bait tool would be incorporated into that of ADS to enable application across large areas and remote landscapes unvaryingly alongside ADS baits. This would minimize any added costs of labor and technology associated with the additional implementation as a control tool and would reduce the amount of labor associated with manually applying the baits across a target landscape.

We examined three types of alternative ground baits that have potential to supplement ADS applications by targeting large, ground foraging BTS; a small rodent (AGB), a rat (XR) and a bird (XB). The small rodent would improve the cost and labor efficiency compared to manual delivery that of XR and XB baits would require. If AGB can target ground foraging snakes as effectively as XR or XB baits, in spite of its smaller size, it is a more cost-effective option to supplement ADS while removing the need for manual distribution of larger ground baits. If, on the other hand, AGB are less effective than the non-AGB baits (XR and XB), then manual distribution of larger ground baits is more advisable.

We sought to identify the bait type with the most potential for improving aerial suppression outcomes. We evaluated efficacies among bait types by modeling bait success rates, or the occurrences of bait fates attributed to a Brown Treesnake, in relation to study site and bait types. We considered non-target species interferences and compared outcomes among study sites. We applied our findings to our subsequent evaluations of the alternative baits in the interest of identifying the most efficient scenario for achieving significant biological effects while minimizing costs. We calculated the Catch Per Unit Effort, or "CPUE". We evaluated the duration of bait persistence using survival analysis. Finally, we proposed three potential management scenarios, which may be applied by wildlife managers toward the objective of landscape-scale Brown Treesnake suppression and long-term ecological restoration.

STUDY AREA

The dataset that we analyzed was produced through a study conducted by the USDA-National Wildlife Research Center (Barrigada, Guam), in collaboration with the Research Corporation of the University of Guam. The island of Guam is the oldest and largest (~540 km^2) of the Marianas Islands. It is situated in the Philippine Sea and is part of Micronesia (13.2 to 13.7EN and 144.6 to 145.0EE). Guam's ecology was characterized by geographical remoteness during its evolutionary history and once consisted of unique endemic biota. Native animals included tropical forest birds, shorebirds, endemic lizards, skinks, and insects. However, native species have largely declined since WWII as invasive and introduced species are increasingly problematic. The island's topography and vegetative cover can be characterized by the substrate type, which is divided from North to South. In the south, volcanic clays support semi-arid highland savannas across rolling mountains, and streams, waterfalls, and some man-made reservoirs persist year-round. In the north, elevated plateaus of limestone forest contain diverse, broad-leaf ever-green tree species. Guam's year-round temperatures fluctuate little (30-31.5 C by day, and 23.5-25 C by night). Rainfall is abundant (>200 cm/year). Seasons are typically divided into *fanuchånan*, or "rainy season" from July to November, and *fañomnåkan*, or "dry season" from February to May. Humidity is higher in rainy season (averaging over 80% humidity) than in the dry season (averaging 75% humidity).

Data was collected from two study sites located at Anderson Airforce Base, a forested area in Northern Guam (Image 1). They are referred to as the HMU (Habitat Management Unit) and the MSA (Munitions Storage Area). The HMU is a 55-ha forested treatment area in Northern Guam where prior studies have been conducted to demonstrate BTS suppression via aerial bait applications using acetaminophen as a BTS pesticides (Dorr et al. 2016, Siers et al. 2020a). The site is surrounded by a snake enclosure, a specially constructed fence which allows snakes to climb out from the inside, but not to enter it. This comprises a population of snakes that is closed to immigration but not to emigration, births, or deaths. The MSA is an adjacent, un-managed forested area that is separated from the HMU by an unpaved road. The HMU is free of wild ungulates and has a understory dense with ferns and native vegetation, whereas the MSA is un-fenced and relatively barren. The HMU underwent ADS treatments during and prior to this study. The MSA received no treatment and was considered a reference study site representative of a Brown Treesnake population that is not affected by aerial suppression.

METHODS

Each of the ground-deployed baits that were tested were larger than the typical bait used in ADS: a large rodent bait (XR, 85-165g), a large bird bait (XB, 85-165g), and an intermediate sized rodent (AGB, 13-17g). The intermediate-sized rodent bait is referred to by the term "Alternative Ground Bait" or AGB. The AGB is larger than the typical "fuzzy" mouse bait used in ADS (4-6g) (Image 2), but small enough to be incorporated into the ADS with minor modifications (Image 3). The rat and chick baits (Image 4) are too large to be deployed via ADS machinery but could be manually dropped from helicopters during ADS treatments. Bait fate observations were obtained using an overhead-mounted game-camera placed over each bait, which recorded photos 24/7 for 72 hours, after which the trial ended (Image 5).

Our dataset consisted of a total sample size of 270 baits, made up of 149 AGB, 60 XR and 61 XB (alternative ground bait, rodent, and bird, respectively). We produced data through the analysis of game-camera images which recorded species interactions for 72 hours for each bait deployed. We defined "bait fate" as the time at which a given bait was no longer available to a foraging Brown Treesnake, and as the category of the bait fate (Brown Treesnake, non-target species interactions, environmental decay, etc.). We calculated the duration of bait availability based on the total time passed between the trial start and the time of bait fate occurrence. The end time was based on the time that bait was considered no longer available. We defined 'non-target' bait faits as those attributed to non-target species, or to environmental degradation. Non-target species included hermit crabs (HCR), Coconut crabs (CCR), toads (TOD) and monitor lizards (MON); bait interactions were counted in these categories if the species visibly consumed the bait on game camera footage.

To control subjectivity in determining when a bait should be defined as 'environmentally degraded' we established a maximum monitoring period. Any bait that remained for >72 hours to be degraded by external environmental factors. This observation period is informed by current management practices, in which baits are recommended to be replaced after 48-72 hours. Baits which were clearly non-viable within the 72-hour observation period were also considered environmentally degraded, often because of consumption by ants or maggots. Baits of unknown fates were not included in this summary (i.e., the bait was dragged out of the camera frame by a hermit crab, so the fate of the bait could not be confirmed). We also excluded two unique instances (one bait-take by a rat and one by a dog) that are not relevant to our analysis.

Statistical analyses were performed in the R environment for statistical computing (R Core Team 2019). The original intent for our data analysis was to continually monitor baits until they were first contacted by a foraging BTS or non-target species, then modeled with “survival” or “time to event” models. These duration times were subjected to survival analysis using Cox proportional hazard model, in order to predict the probability that a bait (AGB, Non-AGB, and overall) remains available at a given time after being deployed.

The frequency at which we observed each bait fate category offered additional insight to trends that vary by the type of bait or by the study site. To evaluate the rate of by bait type, we analyzed bait-fates as binomial count data (0,1) where BTS takes are considered an event (1) and all other outcomes are a non-event (0). We used a general linear model with mixed effects in order to better understand the relationship between successful BTS takes, treated and untreated study sites, and bait types. Fixed effects included bait type and study site; random effects included subplot and transect, in order to account for spatial replication in data collection. There was no temporal replication in the data collected during the ground-bait study, as an event may occur only once per trial period. We performed a Tuki test was performed using the r function ‘emmeans’ to obtain Tukey-adjusted p-values of pairwise comparisons. This method compares the means of every combination of factor levels, or categorical variables, present in the model formula, while penalizing for multiple comparisons to prevent artificial inflation of effect size.

We used the *pairwise* function in R to generate contrasts for all pairwise comparisons. Because of the low sample size, and because we stringently accounted for random effects and multiple comparisons, we considered a meaningful p-value to be 0.10. Alpha values of 0.10 or 0.15 are commonly used in the context of research applied to inform management decisions.

RESULTS

Across all observed data in the treated study site (HMU), the proportion of baits taken by Brown Treesnakes for each bait type was 21.9% of AGB, 15,4% of XB, and 25.9% of XR. In the un-treated reference site, Brown Treesnakes took 41.4% of AGB, 53.6% of XB, and 25% of XR (Figure 1). Brown Treesnakes accounted for 37.8% of all bait fates in the MSA, and 18.5% of bait fates in the HMU. Most remaining bait fates were attributed to environmental degradation. Non-target species interactions accounted for a minority of bait fates across all bait types and study sites except for one; Hermit Crabs removed 42.2% of AGB in the HMU. In comparison, they removed 6.4% of XR and 0% of XR in the HMU; in the MSA there were no observations of Hermit Crab interference in the MSA.

We modeled the bait take rates in relation to bait types and study site (Figure 2). We found that within the HMU there were no significant difference in bait efficacy for any combination of bait types that were compared (P > 0.1 for AGB-XB, AGB-XR, and XB-XR); nor in the MSA P(XB-XR > .05; P(AGB- XR, AGB-XB (P > 0.1). According to model predictions, the probability of baits being taken by snakes in the HMU was lower overall (XB=15.1%, AGB=21.6%, XR=25.8%) compared to the MSA (XB=53.5%, AGB=41.4%, XR=24.8%). Our results demonstrated that all baits have equal probabilities of success in the treated HMU (Tukey-adjusted p-values P> 0.1 in pairwise comparisons).

We plotted mean observed bait durations by bait type and study type (Figure 3). On average, AGB baits lasted roughly .5 days in the HMU and 1.3 days in the MSA, XB baits lasted roughly 1 day in the HMU and 1.5 days in the MSA, and XR baits lasted roughly .75 days in the HMU and .5 days in the MSA. We performed cox proportional hazard analyses and found that across all bait types, the duration of bait availability did not vary significantly by study site ($P>0.1$). According to the model predictions, baits had a 40% probability of persisting until 1 day (24 hours) after deployment. This dropped to approximately 10% probability of survival at approximately 1.5 days (36 hours) after deployment, and 0- 3% probability for 3 days or more (Figure 4).

If AGB can target ground foraging snakes as effectively as XR or XB baits, in spite of its smaller size, it is a more cost-effective option to supplement ADS while removing the need for manual distribution of larger ground baits. If, on the other hand, AGB are less effective than the non-AGB baits (XR and XB), then manual distribution of larger ground baits is more advisable. Having demonstrated that efficacy rates were statistically similar across bait types in the HMU, we grouped baits in terms of their application type instead of the bait type itself. This allows us to compare whether AGB can sufficiently be relied on as a ground bait type, or if more cost-effective methods for implementing XR and XB bait types is worthwhile.

We subjected bait duration to survival analysis using Cox proportional hazard model to predict the probability that a bait (categorized as AGB or Non-AGB) will remain available with increasing time since in the field before they are no longer available to the target species (Figure 5). We found that survival probability did not vary greatly between AGB and non-AGB baits overall ($P > 0.1$). We applied the model predictions to each study site separately. There appeared to we a wider degree of variability in survival rates in the MSA than in the HMU in the plotted predictions, where the survival probabilities for AGB baits decayed more gradually compared to the XB -XR bait group (Figure 6). However, the variance was not significant in either site (P(HMU)=0.15; P(MSA)=0.19).

Finally, estimated bait durations times and success rates for bait types and study sites (Table 2). We calculated the associated "Catch per Unit Effort" (CPUE) as the frequency of success divided by the summed duration of bait availability and multiplied to obtain estimates successes per 100 bait days (Table 2). Across all our data, the summed duration of bait availability was 5,056.5 hours, or 210.68 bait days. Total occurrences of success, or bait fates attributed to Brown Treesnake, was 76. This amounted to a CPUE of 36.07 BTS per 100 bait days for all alternative ground baits tested. CPUE was higher in the number MSA compared to the HMU. The summed duration of medium mouse (AGB) baits was 122.67 bait days (44 bait days in the HMU and 78.7 in the MSA), resulting in a mean CPUE of 32 snakes per 100 bait days (32 in the HMU and 37 in the MSA). We grouped XB and XR baits for management applications reasons stated above. We found that the summed duration of non-AGB availability was 88 days (46.7 in the HMU and 41.3 in the MSA), resulting in a CPUE of 36 snakes per 100 bait days (24 in the HMU, and 53 in the MSA).

DISCUSSION

Our results suggested that the baits demonstrate equal likelihood of success at targeting ground foraging brown Treesnakes. There was no significant variance in efficiencies, which suggests that bird, rat, or medium-sized mouse baits are equally suitable for use as alternative ground baits during aerial treatments. The mouse bait is the most practical for use with the ADS technology. All were widely susceptible to non-target interference. In an un-treated study site, there was slightly more variability in success rates, suggesting that the history of treatment should be taken into consideration when comparing benefits of these bait types in a given scenario.

The variation in bait efficacies by study site could be related to relative snake abundance, or to a wide array of other factors contributing to ecological variability among study sites. For example, Hermit Crabs are much more abundant in the fenced area but are relatively absent in the MSA. The results observed in the HMU may vary if the experiment was repeated in un-fenced, treated landscape, as the presence or absence of ungulates is expected to affect non-target species interferences.

Having demonstrated that efficacy rates were statistically similar across bait types in the HMU, we grouped baits in terms of their application type instead of the bait type itself. This allows us to compare whether AGB can sufficiently be relied on as a ground bait type, or if more cost-effective methods for implementing XR and XB bait types is worthwhile. These results are applicable in cost-benefit analysis for potential management strategies.

Data produced by camera-platform monitoring demonstrated that there was a significant difference in BTS activity between the HMU and MSA during the time of this study. This was reaffirmed during analysis of the alternative ground bait dataset, in which a higher rate of Brown Treesnake- bait interactions were observed in the MSA compared to the HMU.

Based on live lure and camera-platform observations, and the understanding that ADS baits are likely to miss larger BTS, we expect to observe a larger size distribution in the treated HMU than in the untreated reference site, MSA. Unfortunately, there is no SVL data in the alternative ground bait study, nor a way to estimate SVL from game camera photos like in the camera-platform method. A higher size distribution could instead be indicated by bait take patterns.

Because larger BTS are likely to take larger baits, and because smaller BTS are not capable of successfully consuming XB and XR baits, then a higher proportion of large baits should be consumed in the HMU. In the MSA, where no treatment occurred and the size distribution is relatively smaller, a higher portion of smaller bait takes (AGB) would be expected. This pattern was maintained in our observation of the success rates of the AGB and the XR baits. Bait-take rates of the smaller bait (AGB) were significantly higher in the MSA than in the HMU ($P < .05$); this supports either or both assumptions of higher overall BTS activity and a smaller size distribution in the untreated MSA. Of the ground baits that we tested, AGB was the most like ADS baits in terms of size and carrion type. Its smaller size implies that it can be consumed across a wider distribution of snake sizes compared to XR and XB baits. The relatively reduced snake activity with AGB may be considered an additional indicator of overall lower BTS activity that is to be expected in the HMU.

The success rates of large rodent baits (XR) did not significantly differ between sites. Given that MSA has greater overall BTS activity, if there was no difference in size distributions by site, then we would expect to see lower XR takes in the HMU proportionate to difference in AGB takes by site. However, bait-take data indicated a higher proportion of large bait-takes in the HMU relative to the MSA. The pattern suggests that large baits were more likely to be taken by large snakes, and AGB were less likely to be taken by large or small snakes combined. The XR bait demonstrated similar levels of success in the HMU and MSA, despite presumed lower overall BTS activity in the HMU, suggesting the positive effect of the bait withstood the negative effect of treatment in the HMU.

Regardless of whether the BTS activity is higher or lower in other site, our observations suggest that the larger (XR) bait is significantly more successful in the ADS-treated study site, HMU, than the small baits (AGB) that are closer in size to those used in ADS treatments ($P < 0.05$). This provides evidence that XB baits are effectively targeting the BTS that remained in the enclosure throughout multiple ADS treatments, making them an effective tool to increase the efficacy of suppression efforts by supplementing ADS treatments.

Contrary to the pattern stated above, the large rodent (XR) bait-takes were significantly higher in the MSA than in the HMU. This is best explained by their faster decay rate. Based on patterns that we have consistently observed patterns during field work, and anecdotal observations from previous studies, bird baits seem to decompose more quickly than rodent baits of similar size. Due to the higher overall snake activity in the MSA, ground baits of all sizes are likely to be encountered by a foraging BTS sooner than in the HMU. In the HMU, there is a lower likelihood of BTS bait-take in a given amount of time. In the HMU, 24.5% of XR bait fates were 'Environmental Decay', compared to only 18.5% of XR baits in the MSA. Although BTS are expected to prefer avian prey over rodents, the smaller time window of XS bait availability may make them less effective than XR in the treated study site. XR had the lowest rate of environmental decay outcomes (21.8%) and the highest BTS takes (12.7%) in the HMU.

AGB are small and prone to faster decomposition than XR baits; AGB bait fates in the HMU were 20.1% 'environmental decay' and 11.9% hermit crabs. Hermit crabs can be included as a form of environmental decay as detritivores, like ants and maggots. If combined, 32% of AGB bait fates in the HMU were environmental decay. The hermit crab category was kept separate in this comparison simply to point out the interesting difference in hermit crab activity between study sites. Baits, and particularly AGB baits, are highly susceptible to hermit crab consumption in the HMU, which is important to consider over long-term suppression planning that is in place for the HMU.

Regardless of the differences in the fates of each bait type within each study site, our model output indicated that there is no significant differences in the BTS-take rate among bait types in a given site. For the sake of management purposes, it is possible that AGB and XR baits are equally effective in targeting the large BTS that remain on the landscape after ADS treatments. When considering that XR takes were in the HMU equaled those in the MSA, we can infer that XR are the most effective when used in a landscape that has undergone ADS treatments and show the most promise for increasing the probability of targeting larger snakes. Because of their ineffectiveness in the treated landscape, we suggest that XB baits be ruled out as a tool for supplementing ADS treatments.

MANAGEMENT IMPLICATIONS

Based on the objective of long-term suppression efforts that managers plan to continue in the HMU, we propose three potential scenarios for wildlife managers:

1) AGB baits supplement ADS application. PROS: They can be distributed mechanically from the helicopter along with the normal ADS baits but are designed to end up on the forest floor rather than in the trees, where they are more likely to be encountered by (potentially larger) ground-foraging Brown Treesnake. CONS: They persist on the landscape for less time than larger baits, thus increasing the possibility of non-target interference or environmental decay before a BTS encounters the bait. They are particularly susceptible to hermit crab consumption in the HMU, which is important to consider over long-term suppression planning that is in place for the HMU.

2) XR baits supplement ADS applications. PROS: They are the least susceptible of all baits to environmental decay in the treated study site. They have the highest observed likelihood of bait fates that were attributed to Brown Treesnakes in the HMU. Their large size

eliminates the possibility of their being taken by a small size class of snakes, which provides us with the most certainty that Brown Treesnake takes of XR are effectively targeting the large snakes that ADS misses, which is the most important objective of this supplemental tool. CONS: Application of the XR as ground baits requires manual distribution, which may increase labor costs of the suppression efforts. The HMU is accessible by foot, but this may be less desirable in future scenarios if ADS treatments are applied on more remote and rugged terrains. The shipping cost and price of the bait itself may be higher than AGB.

3) A combination of both ABG and XR baits be used. The decision of whether to include AGB, XR or both baits will depend on their cost effectiveness- an assessment which is beyond the scope of this study. Is the cost of bait preparation worth the probability of snake mortalities that the bait provides? Even if a ground bait has a low likelihood of being taken by a BTS relative to the arboreal ADS baits, increasing the chances of targeting large BTS, which are the most important contributors to population growth, may be invaluable.

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Note that the figures, Tables, and Images are not included in this document, as per WMB submission guidelines. WMB guidelines do require figures and table captions to be included alone at the end of the manuscript, however they are not included here to reduce redundancy throughout the thesis version of this manuscript. Figures and tables mentioned in this section can be found with their associated captions in Appendix B of Chapter 3.

Chapter 3:

Supplementary Images, Figures & Tables

Figures and tables are presented separately from the text, as per submission format guidelines for the Journal of Wildlife Management (Chapter 2A), and for the Wildlife Society Bulletin: Tools and Technology (Chapter 2B)

Appendix A:

Supplementary Images, Tables and Figures

Evaluation of landscape-scale BTS suppression and a novel monitoring method

Figures and tables are presented separately from the text, as per submission format guidelines for the Journal of Wildlife Management: Research Articles

TABLES

HMU Nightly Snake Detection Rates

	Dependent variable:	
	BTS	
	(1)	(2)
Night	0.070***	0.266***
	(0.010)	(0.044)
I(Night2)		-0.013***
		(0.003)
Constant	-0.001	-0.539***
	(0.131)	(0.177)
Observations	1,109	1,109
Log Likelihood	-1,982.637	-1,972.294
Akaike Inf. Crit.	3,973.274	3,954.588
Bayesian Inf. Crit.	3,993.319	3,979.644
Note:	*p<0.1; **p<0.05; ***p<0.01	

Table 1a) We modeled nightly variation in snake detection rates during a 14-night trial across all data collected in the treated study site, HMU. We compared models with and without a quadratic term for trial night (*Night^2)* (Model 2). Both models included a continuous term for Trial Night, and the study Trial as a random effect to account for spatial replication in data collection across multiple nights. We found that both terms were significant, and that Model 2 had a lower AIC value. The columns list the regression coefficient (β) for each predictor term, with asterisks to indicate the significance of the p-value (*), and the standard error in parentheses below.

Trial Night & Site Model Comparisons

	Dependent variable:	
	BTS	
	(1)	(2)
SiteMSA	1.000***	1.000***
	(0.100)	(0.200)
Night	0.200***	0.200***
	(0.030)	(0.040)
NightSQ	-0.010***	-0.010***
	(0.002)	(0.002)
SiteMSA:Night		-0.010
		(0.020)
Constant	-0.400***	-0.400***
	(0.200)	(0.200)
Observations	1,512	1,512
Log Likelihood	-2,975.000	-2,974.000
Akaike Inf. Crit.	5,966.000	5,967.000
Bayesian Inf. Crit.	6,008.000	6,015.000
Note:	*p<0.1; **p<0.05; ***p<0.01	

Table 1b) We modeled snake detection rates in relation to trial night and study site to distinguish between un-controlled temporal variability and treatment effect in the HMU. We included a continuous and a quadratic term for Trial Night (Model 1). We tested an interactive term (Trial Night*Study Site), which was not significant (Model 2). We found that Model 1 exhibited the best fit. For both models the trial night (including both a continuous and quadratic term) and study site were significant predictors of nightly contact rates (P<0.01 for all). The columns list the regression coefficient for each predictor term, with asterisks to indicate the significance of the p-value (*), and the standard error in parentheses below.

Mean Detection Rates by Site

	Site	Observed	Predicted
1	HMU	1.88	1.85
2	MSA	4.05	4.06

MSA: Detection Rates by Trial Night

	Night	Observed	Predicted
1	1.00	2.10	2.22
2	2.00	3.36	2.71
3	3.00	4.05	3.21
4	4.00	3.43	3.73
5	5.00	3.82	4.21
6	6.00	4.62	4.66
7	7.00	4.51	5.09
8	8.00	4.69	5.13
9	9.00	4.86	5.28
10	10.00	4.31	5.43
11	11.00	4.64	5.41
12	12.00	6.35	5.56
13	13.00	6.12	5.27
14	14.00	5.08	4.86

HMU: Detection Rates by Trial Night

	Night	Observed	Predicted
1	1.00	0.83	0.94
2	2.00	1.00	1.14
3	3.00	1.51	1.39
4	4.00	1.77	1.60
5	5.00	1.95	1.71
6	6.00	1.93	1.81
7	7.00	2.24	1.96
8	8.00	2.09	2.25
9	9.00	2.08	2.33
10	10.00	2.43	2.40
11	11.00	2.32	2.38
12	12.00	2.21	2.31
13	13.00	2.41	2.17
14	14.00	2.12	2.19

Table 1c) We evaluated the predicted and observed nightly snake encounter rates by study site (Above); across all trial nights, expected mean detection rates were 4.06 in the MSA and 1.88 in the HMU. We broke down observed and predicted detection rates by trial night and study site (Below); in both sites, detection rates slowly increase over the first few nights of trial then level off. We generated model predictions with a negative binomial mixed-effects model: *BTS~Site+Night+Night^2+(1|Trial).* We found that detection rates were significantly higher in the untreated site (MSA) compared to the treated site (HMU) ($P<.001$), and that detection rates for both sites varied significantly by trial night ($P<.001$).

Temporal Trends: Snake REcovery in the HMU

	Dependent variable:	
	BTS	
	(1)	(2)
CDate	0.006***	-2.699***
	(0.001)	(0.819)
CDate_2		0.0001***
		(0.00002)
Constant	-118.464***	24,910.010***
	(20.643)	(7,574.988)
Observations	626	626
Log Likelihood	-1,021.866	-1,016.437
Akaike Inf. Crit.	2,053.732	2,044.874
Bayesian Inf. Crit.	2,075.929	2,071.511
Note:	*p<0.1; **p<0.05; ***p<0.01	

Table 2a) We evaluated snake detection rates in the HMU in relation to increasing time comparisons since the last treatment application (here termed "CDate). We also tested an additional quadratic term for CDate (Model 2). In both models, we included random effects of (Site|Trial), Night and Night^2. We found that Model 2 had a lower AIC value, and both fixed effects terms were highly significant in (P < 0.01). The model of best fit is BTS ~ Time Since ADS + Time Since ADS^2 + (1|Night) + (1|Night^2) + (Site|Trial). The columns list the regression coefficient for each predictor term, with asterisks to indicate the significance of the p-value (*), and the standard error in parentheses below.

BTS~ Site + Treatment + (Site*Treatment) + (1|Night)+ (1|Night^2)

Temoral Trends: Study Site Comparison

	Dependent variable:	
	BTS	
	(1)	(2)
CDate	-3.000***	-2.000***
	(0.700)	(0.700)
CDate_2	0.0001***	0.0001***
	(0.00002)	(0.00002)
SiteMSA	1.000***	150.000***
	(0.100)	(43.000)
CDate:SiteMSA		-0.008***
		(0.002)
Constant	29,091.000***	21,438.000***
	(6,248.000)	(6,592.000)
Observations	946	946
Log Likelihood	-1,798.000	-1,792.000
Akaike Inf. Crit.	3,610.000	3,601.000
Bayesian Inf. Crit.	3,644.000	3,640.000
Note:	*p<0.1; **p<0.05; ***p<0.01	

Table 2b) We compared camera platform observations from both study sites to distinguish environmental stochasticity (represented in the reference site, MSA) from the effects of treatment in the HMU. In addition to continuous and quadratic terms for Time Since Treatment (CDate and CDate_2), we include terms for study site and an interactive term (CDate * Site), to allow temporal trends to vary by treated and untreated study site. We found that the interactive model (Model 2) improved the AIC value compared to the non-interactive model. Model 2 had the lowest AIC value and all terms were significant. The columns list the

regression coefficient for each predictor term, with asterisks to indicate the significance of the p-value (*), and the standard error in parentheses below.

Before-After Treatment Comparison

	Dependent variable:
	BTS
SiteMSA	0.400^{***}
	(0.100)
ADSAfter	-0.300^{**}
	(0.100)
SiteMSA:ADSAfter	0.900^{***}
	(0.200)
Constant	0.800^{***}
	(0.100)
Observations	599
Log Likelihood	-1,241.000
Akaike Inf. Crit.	2,496.000
Bayesian Inf. Crit.	2,527.000
Note:	*p<0.1; **p<0.05; ***p<0.01

Table 3a) We compared camera platform observations from both study sites to distinguish environmental stochasticity (represented in the reference site, MSA) from the effects of treatment in the HMU. We compared data that represented time periods before and after treatment applications. We included a fixed effect for treatment status (here termed "ADS"), categorized as before or after treatment and an interactive term for Site*Treatment Status to allow responses to vary in treated and untreated study sites. Study site was highly significant *(*P< .01, β (MSA) =.400, SE (MSA) =.10); Treatment effect was significant *(*P< .01, β (After) = -

.300, SE=.10); and the interactive term was highly significant *(*P(MSA* After ADS)< .01, β =.90, SE=.20). Our "before treatment" data was collected after several months without treatment, at which point snake abundance had increased in the HMU, however mean detection rates in the "before" treatment group were still lower in the HMU on average. The columns list the regression coefficient for each predictor term, with asterisks to indicate the significance of the p-value (*), and the standard error in parentheses below.

BTS~ Treatment + Site + (Treatment * Site) + (1|Night) + (1|Night^2)

Treatment-Study Site Pairwise Comparison

	Site	ADS	contrast	response	SE	df	asymp.LCL	asymp.UCL	null	z.ratio	p.value
1	HMU	Before	.	2.17	0.22	Inf	1.67	2.82	1.00	7.77	0.00
2	HMU	After	.	1.55	0.20	Inf	1.10	2.18	1.00	3.41	0.00
3	MSA	Before	.	3.31	0.42	Inf	2.36	4.64	1.00	9.36	0.00
4	MSA	After	.	5.74	0.78	Inf	4.01	8.22	1.00	12.87	0.00
5	HMU	.	Before - After	1.40	0.18	Inf	0.99	1.97	1.00	2.57	0.06
6	MSA	.	Before - After	0.58	0.09	Inf	0.38	0.87	1.00	-3.56	0.00

Table 3b) We evaluated the relationship between detection rates by study site and treatment status. We performed pairwise comparisons based on a mixed-effects model (Table 3a) using the *emmeans* function in R to compare all combinations of factor levels. We back transformed the model predictions to the response variable, nightly snake detection rates. In the HMU, expected mean snake detection rates were 2.0 before and 1.55 after treatment. In the MSA, expected mean detection rates were 3.25 before and 5.76 after treatment. Column "Site" indicates Study Site and column "ADS" indicates Treatment Status (before or after treatment).

Effects of Repeated Treatments in the HMU

	Dependent variable:
	BTS
ADSTreatment 2	0.060
	(0.100)
ADSTreatment 3	-0.500***
	(0.100)
Constant	0.900***
	(0.100)
Observations	455
Log Likelihood	-927.000
Akaike Inf. Crit.	1,866.000
Bayesian Inf. Crit.	1,891.000
Note:	*p<0.1; **p<0.05; ***p<0.01

Table 3c) We compared snake detection rates in the HMU that were observed before, between and after two successive aerial treatments (here termed ADS Treatment 1, ADS Treatment 2, and ADS Treatment 3). We found that detection rates did not significantly vary after in Treatment Period 2, after one ADS application (P(Treatment 2) > 0.1, β =.06, SE =.10); detection rates significantly decreased after two treatment applications, in the following treatment period (P(Treatment 3) < .01, β= - 0.50, SE =.10). We used a negative binomial mixed-effects model with Treatment Status as a fixed effect. Mixed effects no longer included "Trial" because of the limitations in the number of trials included in the subset of data analyzed by this model. The columns list the regression coefficient for each predictor term, with asterisks to indicate the significance of the p-value (*), and the standard error in parentheses below.

Observed Detection Rates by Treatment (HMU)

Status	min	median	mean	max	SE	n
Treatment 1	0	2	2.77	11	0.244	105
Treatment 2	0	3	3.04	10	0.215	171
Treatment 3	0	1	1.89	11	0.203	104

Treatment-Study Site Pairwise Comparison

	ADS	contrast	response	SE	df	asymp.LCL	asymp.UCL
1	Treatment 1	.	2.52	0.33	Inf	1.78	3.56
2	Treatment 2	.	2.67	0.31	Inf	1.97	3.62
3	Treatment 3	.	1.56	0.22	Inf	1.08	2.25
4	.	Treatment 1 - Treatment 2	0.94	0.12	Inf	0.67	1.32
5	.	Treatment 1 - Treatment 3	1.62	0.24	Inf	1.09	2.40
6	.	Treatment 2 - Treatment 3	1.71	0.23	Inf	1.20	2.45

Table 3d) (*Above):* We evaluated mean nightly snake detection rates in the HMU during two successive aerial treatment applications (Treatment 1=before, Treatment 2=between, Treatment 3=after). *(Below):* We modeled mean nightly contact rates in relation to treatment applications in the HMU. Column "Response" indicates model predictions back transformed into the response variable, nightly snake detection rates. We found that in the HMU, expected nightly snake detection rates were 2.52 before, 2.67 between, and 1.71 after the two treatments. We performed pairwise comparisons based on a mixed-effects model (Table 3c) using the *emmeans* function in R to compare all combinations of factor levels. We produced contrasts for each possible comparison of Treatment Status (ADS).

	Dependent variable:
	BTS
Subplot2	-0.790***
	(0.180)
Subplot3	0.170
	(0.150)
Subplot4	-0.370**
	(0.160)
Subplot5	0.180
	(0.150)
ADSTreatment 2	0.140
	(0.120)
ADSTreatment 3	-0.410***
	(0.140)
Constant	0.940***
	(0.160)
Observations	455
Log Likelihood	-907.000
Akaike Inf. Crit.	1,834.000
Bayesian Inf. Crit.	1,875.000
Note:	*p<0.1; **p<0.05; ***p<0.01

Table 4a) We evaluated spatial heterogeneity in HMU snake detection rates across three treatment periods (before, between and after two successive aerial treatment applications). We found that the interactive model had a marginally lower AIC value (1,830) compared to the non-interactive model (AIC=1,834). The interactive model showed only two significant subplot-treatment interactions; these were for Subplot 2 during Treatment 2 (P<0.1, β = .880, SE=.47), and Subplot 3 during Treatment 3 (P> .05, β = 1 .880, SE= .4). We did not include the interactive model output here, but we followed this model evaluation with pairwise comparisons based on the interactive model.

Treatment Effects on Spatial Heterogeneity in Detection Rates

Subplot = 1:

contrast	estimate	SE	df	z.ratio	p.value
Treatment 1 - Treatment 2	-0.17	0.24	Inf	-0.700	0.7500
Treatment 1 - Treatment 3	0.17	0.28	Inf	0.600	0.8100
Treatment 2 - Treatment 3	0.34	0.26	Inf	1.300	0.3700

Subplot = 2:

contrast	estimate	SE	df	z.ratio	p.value
Treatment 1 - Treatment 2	-1.06	0.40	Inf	-2.600	0.0200
Treatment 1 - Treatment 3	-0.61	0.45	Inf	-1.400	0.3500
Treatment 2 - Treatment 3	0.44	0.33	Inf	1.300	0.3800

Subplot = 3:

contrast	estimate	SE	df	z.ratio	p.value
Treatment 1 - Treatment 2	0.16	0.23	Inf	0.700	0.7700
Treatment 1 - Treatment 3	1.05	0.29	Inf	3.600	<.0001
Treatment 2 - Treatment 3	0.89	0.27	Inf	3.300	<.0001

Subplot = 4:

contrast	estimate	SE	df	z.ratio	p.value
Treatment 1 - Treatment 2	-0.70	0.29	Inf	-2.400	0.0400
Treatment 1 - Treatment 3	0.23	0.36	Inf	0.600	0.8000
Treatment 2 - Treatment 3	0.93	0.30	Inf	3.100	0.0100

Subplot = 5:

contrast	estimate	SE	df	z.ratio	p.value
Treatment 1 - Treatment 2	0.32	0.24	Inf	1.300	0.4000
Treatment 1 - Treatment 3	0.54	0.28	Inf	1.900	0.1400
Treatment 2 - Treatment 3	0.22	0.26	Inf	0.900	0.6600

Results are given on the log (not the response) scale.
P value adjustment: tukey method for comparing a family of 3 estimates

Table 4b) We evaluated variation in detection rates by subplot before, between and after successive aerial treatments (here termed ADS Treatment 1, ADS Treatment 2, and ADS Treatment 3). We generated contrasts for all combinations of subplot in relation to treatment applications (Treatment 1=before, Treatment 2=between, Treatment 3=after the two successive aerial treatment applications). We found that detection rates responded differently to treatments among subplots.

We performed pairwise comparisons based on a mixed-effects model (Table 3c) using *emmeans* function in R to compare all factor level combinations.

Mean Estimated SVL by Study Site

Site	min	median	mean	max	SE	n
HMU	767	1261	1271	1953	4.83	1194
MSA	529	1170	1172	1897	4.22	750

Table 5a) We estimated snake snout-vent-length (SVL) from game camera images to evaluate differenced mean snake size by study site. We compared mean SVL by study site. We found that the average snout-vent length (SVL) in the treatment site (HMU) was greater than the average snake SVL in the un-treated reference site (MSA). In the HMU, the mean SVL was 1271.5 mm; in the MSA, the mean SVL was 1171.5mm.

Snake Size by Study Site

	Dependent variable:
	SVL
SiteMSA	-0.086^{***}
	(0.009)
Constant	7.200^{***}
	(0.015)
Observations	2,809
Log Likelihood	-18,071.000
Akaike Inf. Crit.	36,154.000
Bayesian Inf. Crit.	36,189.000
Note:	*p<0.1; **p<0.05; ***p<0.01

Table 5b) We evaluated the relationship between snake size and study site using a negative binomial mixed-effects model. We used Site as a fixed effect and (Site|Subplot) as random effects. We found that the estimated snake size (snout-vent length, or SVL) varied significantly by Study Site ($P<.001$, $\beta(MSA) = -.086$, $SE= .009$).

SVL Before, Between, After Treatments (HMU)

Treatment	min	median	mean	max	SE	n
Treatment 1	964	1301	1313	1835	12.4	167
Treatment 2	790	1237	1243	1724	8.89	327
Treatment 3	819	1260	1268	1952	12.0	133

Table 5c) We estimated snake snout-vent-length (SVL) from game camera images to evaluate differenced mean snake sizes in the HMU before, between and after successive aerial treatments (here termed ADS Treatment 1, ADS Treatment 2, and ADS Treatment 3). Based on observed data, we found that SVL was 1,313mm before, 1,243 mm between, and 1,268mm after the two aerial treatments.

SVL by Treatment Status in the HMU

	Estimate	Std. Error	z-value	Pr(>|z|)
(Intercept)	7.180047	0.009476	757.720	< 2e-16 ***
Treatment 2	-0.054789	0.011652	-4.702	2.58e-06 ***
Treatment 3	-0.034596	0.014239	-2.430	0.0151 *

Signif. codes: 0 '***'0.001 '**'0.01 '*'0.05 '.' 0.1 ' '1

Theta: 70.26 Std. Err.: 4.18 2 x log-likelihood: -8099.85

Table 5d) We used a negative binomial mixed-effects model to evaluate snake snout-vent-length (SVL) in relation to successive treatment applications in the HMU. We used Treatment Status as a fixed effect and grouped data that occurred before, between or after the two treatment applications (here termed ADS Treatment 1, ADS Treatment 2, and ADS Treatment 3). We found that the expected SVL was 1,313mm before, 1,243 mm between, and 1,268mm after the two aerial treatments. We found that SVL variance was highly significant from Treatment Periods 1 to 2 ($P<.001$, $\beta(2) = -.055$, $SE= .009$), and was significant from Treatment Period 1 to 3 ($P< .05$, $\beta(3) = -.03$, $SE= .014$).

Methods Comparison: Negative Binomial Distribution

	Dependent variable:		
	BTS		
	(1)	(2)	(3)
Methodcameraplatform	-0.520**	-0.600***	-0.600***
	(0.220)	(0.220)	(0.220)
Constant	0.800***	0.680***	0.680***
	(0.120)	(0.140)	(0.140)
Observations	819	819	819
Log Likelihood	-1,364.000	-1,351.000	-1,351.000
Akaike Inf. Crit.	2,740.000	2,715.000	2,717.000
Bayesian Inf. Crit.	2,769.000	2,748.000	2,755.000
Note:	*p<0.1; **p<0.05; ***p<0.01		

Table 6a) We modeled the relationship between snake detection rates and method type with subplot as a random effect, nested by monitoring method. We included time since treatment as a random effect in model 2; in model 3 we included a continuous and quadratic term for time since treatment. We found that Model 2 had a slightly lower AIC value compared to Model 3. We found that the effect of the monitoring method was highly significant (P<.01, β(Camera Platform) = -600, SE= .220). Observations occurred during the ~9-month period after treatments stopped in the HMU, during which we detected a significant recovery in snake detection rates in camera-platform data (Table 2a). The dataset used for this model included snake detection data pooled for both monitoring methods (bait tube and camera platform). Bait-take rates were considered as count data rather than binomial response by summing the number of occurrences, or bait takes, for each trial, subplot and transect. The maximum number of baits available per transect was 10, however bait takes never exceeded this number. Data for snake detection rates was over dispersed (Variance/Mean > 1), so a negative binomial distribution was used.

Monitoring Method Comparison

	Method	Month	mean	SE	n
1	Bait Tube	April	1.35	0.28	20.00
2	Bait Tube	May	1.25	0.19	28.00
3	Bait Tube	June	1.00	0.22	16.00
4	Bait Tube	July	0.81	0.23	16.00
5	Bait Tube	August	1.17	0.21	12.00
6	Bait Tube	September	3.00	0.34	16.00
7	Bait Tube	October	2.75	0.30	16.00
8	Bait Tube	November	2.31	0.37	16.00
9	Bait Tube	December	2.05	0.22	25.00
10	Camera Platform	April	0.35	0.09	88.00
11	Camera Platform	May	0.89	0.16	115.00
12	Camera Platform	June	2.07	0.26	67.00
13	Camera Platform	July	0.82	0.18	74.00
14	Camera Platform	August	0.86	0.19	78.00
15	Camera Platform	September	1.51	0.21	78.00
16	Camera Platform	October	1.74	0.62	19.00
17	Camera Platform	November	2.54	0.31	67.00
18	Camera Platform	December	3.29	0.49	38.00

Table 6b) We compared mean monthly detection rates that were observed by two monitoring methods (camera platform and bait tube). With bait tube monitoring data, we detected a 51.9 % increase in detection rates from April (mean= 1.35, SE= .28) to December (mean= 2.05, SE= .222) of 2020. With camera platform data we detected an 840 % increase in detection rates from April (mean= 0.35, SE= .09) to December (mean= 3.29, SE= .49) of 2020. When plotted, data from both methods demonstrated a similar trend of increasing detection rates (Figure 6a, 6b). Observations occurred during the ~9-month period after treatments stopped in the HMU, during which we detected a significant recovery in snake detection rates in camera-platform data (Table 2a). Bait-take rates were considered as count data rather than binomial response by summing the number of occurrences, or bait takes, for each trial, subplot and transect. The maximum number of baits available per transect was 10, however bait takes never exceeded this number.

Snake Detection Rates~ Month * Method+ (Method|Plot)

	Chisq	Df	Pr(>Chisq)
Method	0.79	1.00	0.37
Month	130.94	8.00	0.00
Method:Month	32.45	8.00	0.00

Table 6c) We modeled the relationship between snake detection rates by month and method of data collection using a mixed-effects model with a negative binomial distribution. We found that detection rates did not vary by monitoring method (P >.05) but they did vary significantly by Month (P<.001), and that there was a significant interaction between Method and Month (P<.001).

Pairiwse Comparison: Method Detections by Month

	contrast	Month	estimate	SE	df	asymp.LCL	asymp.UCL	z.ratio	p.value
1	Bait Tube effect	April	0.69	0.20	Inf	0.24	1.14	3.41	0.00
2	Camera Platform effect	April	-0.69	0.20	Inf	-1.14	-0.24	-3.41	0.00
3	Bait Tube effect	May	0.27	0.17	Inf	-0.11	0.65	1.58	0.11
4	Camera Platform effect	May	-0.27	0.17	Inf	-0.65	0.11	-1.58	0.11
5	Bait Tube effect	June	-0.36	0.21	Inf	-0.83	0.12	-1.69	0.09
6	Camera Platform effect	June	0.36	0.21	Inf	-0.12	0.83	1.69	0.09
7	Bait Tube effect	July	-0.02	0.22	Inf	-0.52	0.48	-0.10	0.92
8	Camera Platform effect	July	0.02	0.22	Inf	-0.48	0.52	0.10	0.92
9	Bait Tube effect	August	0.16	0.23	Inf	-0.35	0.67	0.69	0.49
10	Camera Platform effect	August	-0.16	0.23	Inf	-0.67	0.35	-0.69	0.49
11	Bait Tube effect	September	0.32	0.18	Inf	-0.09	0.73	1.72	0.08
12	Camera Platform effect	September	-0.32	0.18	Inf	-0.73	0.09	-1.72	0.08
13	Bait Tube effect	October	0.15	0.22	Inf	-0.35	0.64	0.67	0.50
14	Camera Platform effect	October	-0.15	0.22	Inf	-0.64	0.35	-0.67	0.50
15	Bait Tube effect	November	-0.09	0.19	Inf	-0.51	0.33	-0.47	0.64
16	Camera Platform effect	November	0.09	0.19	Inf	-0.33	0.51	0.47	0.64
17	Bait Tube effect	December	-0.30	0.18	Inf	-0.70	0.09	-1.72	0.09
18	Camera Platform effect	December	0.30	0.18	Inf	-0.09	0.70	1.72	0.09

Table 6d) We modeled snake detection rates in relation to the Month and the Method of data collection. We found that monitoring method effect was not significant (P >.05), Month was significant (P<.001), and that the interaction between the terms was significant (P<.001) (Table

6c). Contrasts showed that monthly detection rates did not vary significantly between the two monitoring methods for 8 of the 9 months (P(April) > .05). This suggests that detection rates for both methods vary similarly in response to significant variance in relative snake abundance. Contrasts were based on a negative binomial mixed-effects model (Table 6c).

Pairiwse Comparison: Method Detections by Month

	contrast	Method	estimate	SE	df	asymp.LCL	asymp.UCL	z.ratio	p.value
1	April effect	Bait Tube	-0.159	0.256	Inf	-0.869	0.550	-0.623	0.533
2	May effect	Bait Tube	-0.236	0.226	Inf	-0.862	0.390	-1.047	0.380
3	June effect	Bait Tube	-0.460	0.304	Inf	-1.301	0.382	-1.514	0.245
4	July effect	Bait Tube	-0.667	0.321	Inf	-1.559	0.224	-2.075	0.114
5	August effect	Bait Tube	-0.305	0.332	Inf	-1.227	0.617	-0.918	0.403
6	September effect	Bait Tube	0.639	0.244	Inf	-0.039	1.317	2.615	0.080
7	October effect	Bait Tube	0.552	0.247	Inf	-0.134	1.238	2.232	0.114
8	November effect	Bait Tube	0.379	0.254	Inf	-0.326	1.083	1.491	0.245
9	December effect	Bait Tube	0.258	0.215	Inf	-0.339	0.855	1.198	0.346
10	April effect	Camera Platform	-1.357	0.193	Inf	-1.893	-0.821	-7.019	0.000
11	May effect	Camera Platform	-0.597	0.159	Inf	-1.038	-0.156	-3.754	0.000
12	June effect	Camera Platform	0.434	0.136	Inf	0.056	0.813	3.181	0.003
13	July effect	Camera Platform	-0.444	0.157	Inf	-0.881	-0.008	-2.823	0.006
14	August effect	Camera Platform	-0.439	0.152	Inf	-0.861	-0.018	-2.889	0.006
15	September effect	Camera Platform	0.187	0.133	Inf	-0.182	0.557	1.407	0.160
16	October effect	Camera Platform	0.434	0.253	Inf	-0.267	1.136	1.717	0.097
17	November effect	Camera Platform	0.735	0.131	Inf	0.372	1.098	5.619	0.000
18	December effect	Camera Platform	1.047	0.157	Inf	0.611	1.482	6.660	0.000

Table 6e) We modeled snake detection rates in relation to the Month and the Method of data collection. We found that method was not significant (P >.05), Month was significant (P<.001), and that there was significant interaction between the terms (P<.001) (Table 6c). We performed pairwise comparisons to produce contrasts of monthly change by method type. We found that camera platform data detected significant variance between 7 of 9 successive months (P(September, October) > .05) and that bait tube data detected no significant variation by month

(P > .05) (Figure 6e). Camera-platform monitoring detected a continuous increase in snake detection rates with increasing time since last treatment, but bait tube detected a decrease from September to December, similar to the trend that was observed in the un-treated reference site (Objective 2). Contrasts were based on a negative binomial mixed-effects model with interactive fixed effects for Method and Month, and a random effect for subplot nested by method type (Table 6c).

FIGURES

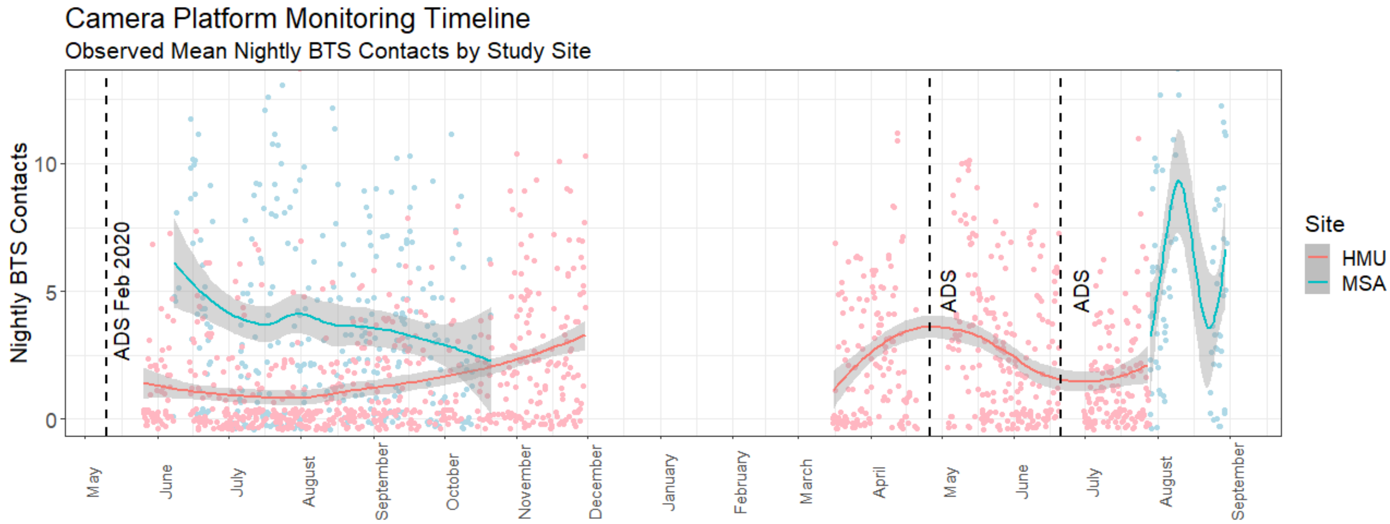


Figure 1) A timeline of Camera platform monitoring. Data collection occurred from June 2020 until September of 2021, with a three month pause in data collection from December 2020-March 2021. Our analysis included a cumulative 12 months of data, comprised of 23 trials completed in the HMU (red) and 12 trials completed in the MSA (blue). Points indicate nightly snake detection counts observed with the camera platform monitoring method. The black, dashed lines indicate when aerial toxic bait treatments applied in the HMU via the aerial delivery system (ADS). An early ADS treatment occurred in February of 2020, prior to the commencement of camera platform data collection. There was an unanticipated pause in treatments with the onset of the COVID-19 Pandemic, and treatments did not recommence until 2021. Camera platform monitoring continued, except for a pause from December 2020-March 2021.

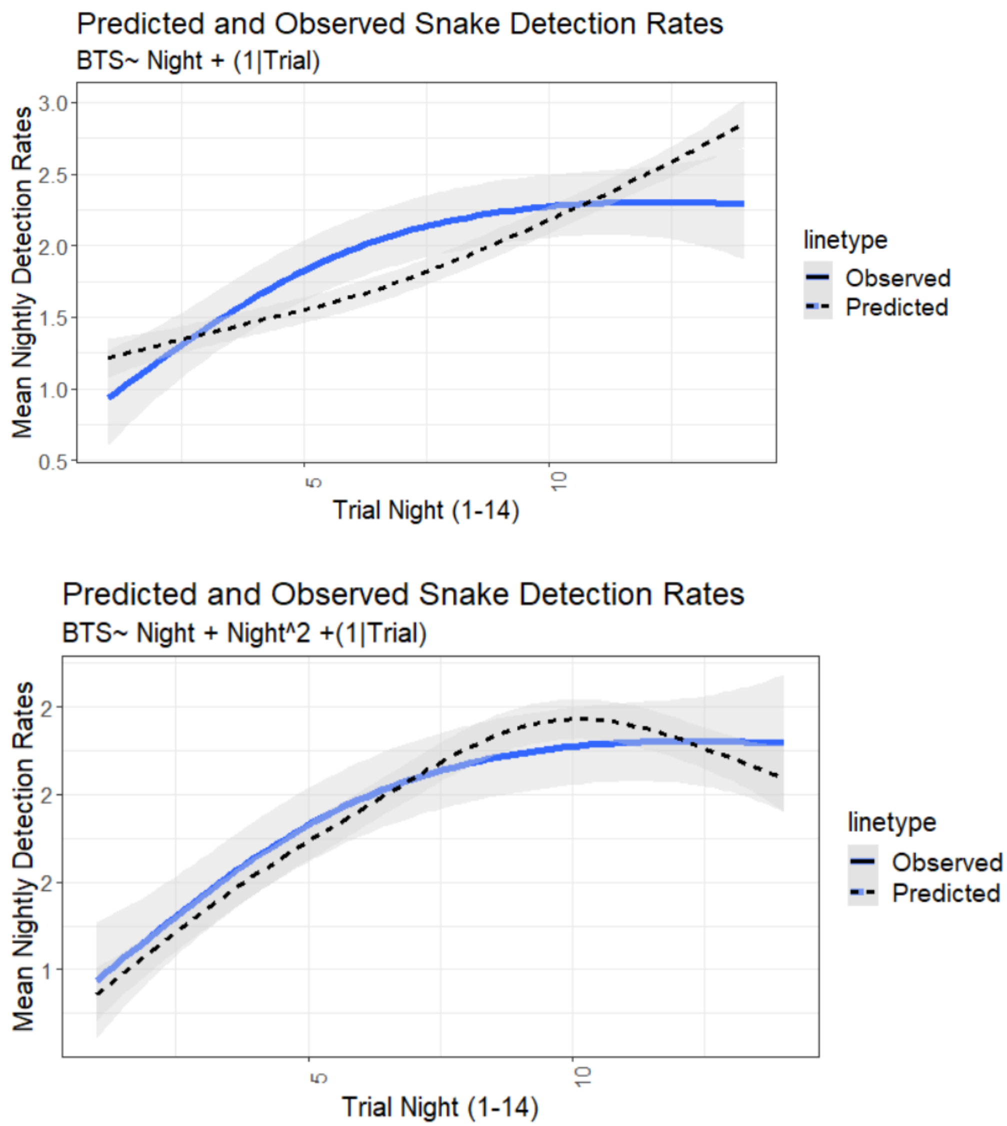


Figure 1a) We plotted predicted and observed snake detection rates by trial night for all fata collected in the treated study site, HMU. WE used a negative-binomial mixed effects model the relationship between snake detection rates and the trial night during a 14-night trial *(top)*, and we also tested a continuous and quadratic term for trial night (Night^2) *(below)*. The dashed line indicates model predictions while the black line indicated observed nightly detection rates with smoothing applied using locally weighted means (loess). The shaded areas indicate confidence intervals (CI=0.95). The model fit was greatly improved when we

included the quadratic term, illustrating an increase detection rates that levels off during the first few nights in a trial.

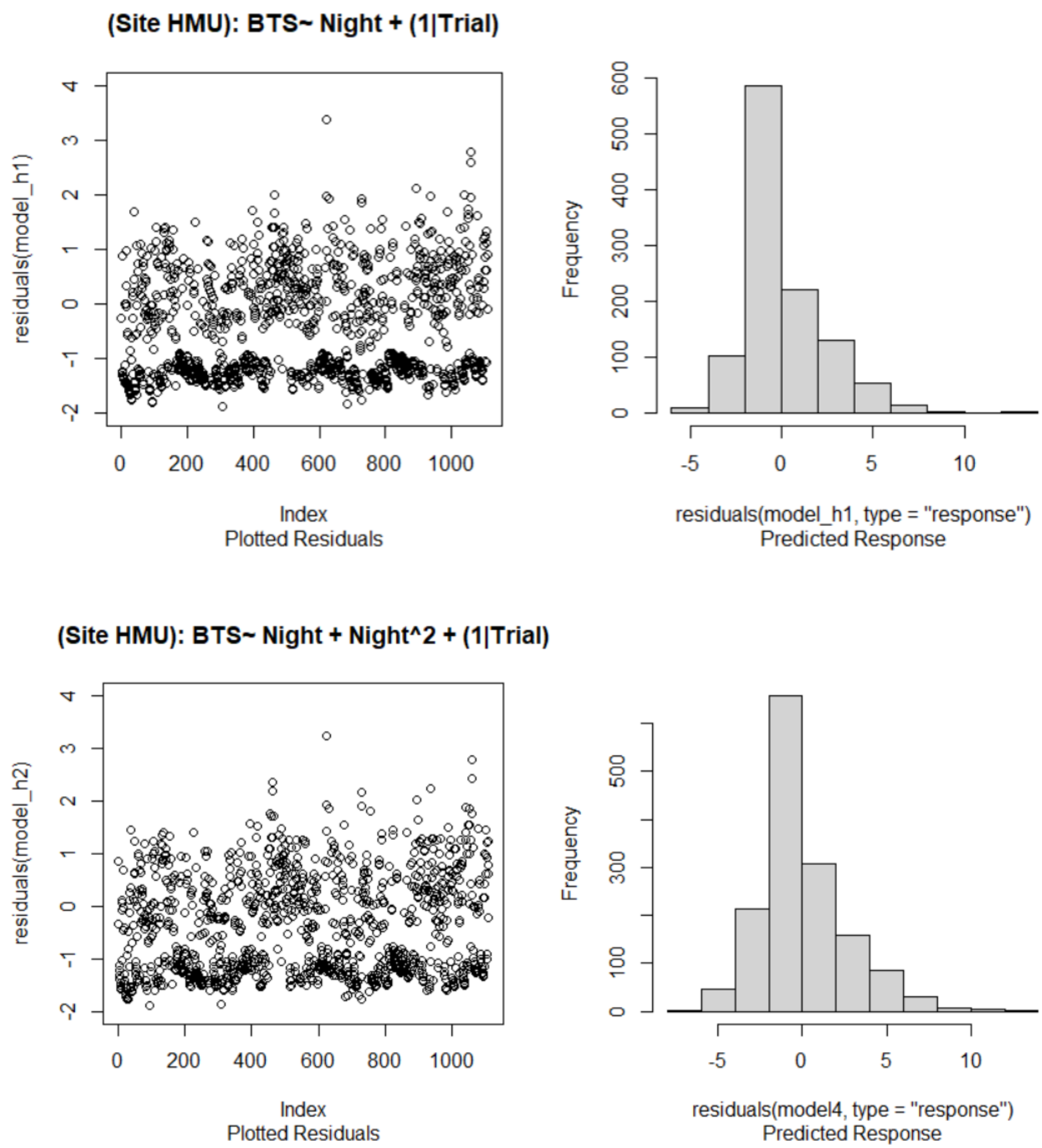


Figure 1b) We modeled snake detection rates in relation to the trial night (nights 1-14). We plotted the residual plots for our negative binomial models, which includes data from the treated

study site, HMU. *(Top)* Model terms include fixed effects for *Night* and the random effect *(1|Trial*) (Model 1, Table 1a). (*Below)* Model terms include fixed effects for *Night and Night^2* and the random effect *(1|Trial*) (Model 2, Table 1a). The fitted residuals illustrate that a majority of the residual values lie between y= -2 and y= 2, indicating a typical distribution pattern for a negative binomial regression; (*Left*) Points indicate the plotted residuals; (*Right)* a Histogram of predicted response residuals.

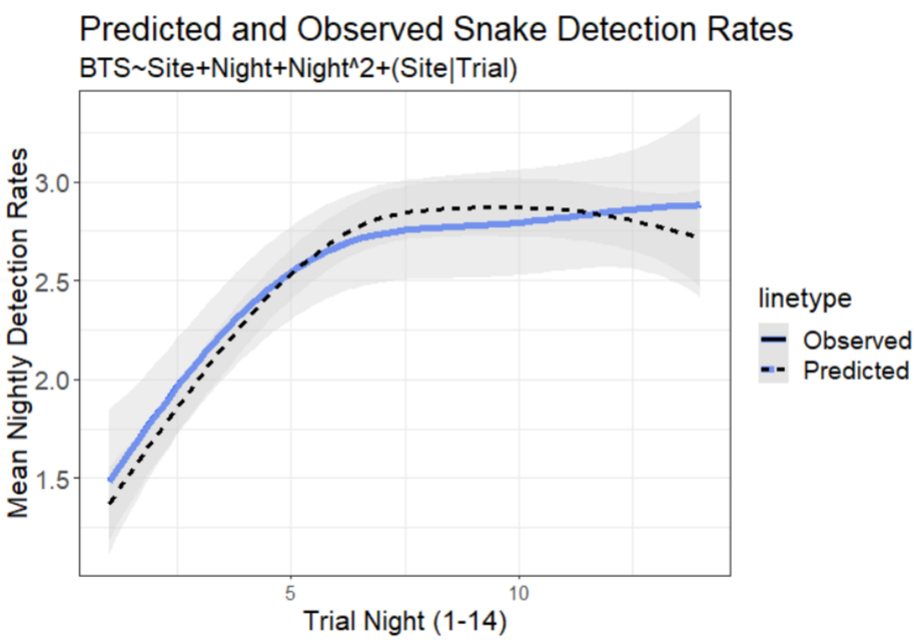


Figure 1c) We plotted the predicted and observed mean snake detection rates during a 14-night trial. The black dashed line indicates model predictions for all data, grouped across both study sites (Model 2, Table 1b). The blue line indicates observed data. Terms for Study Site, Night, and Night^2 were all significant (P<.01). Grey shaded areas indicate confidence intervals (CI=0.95). Smoothing was applied using local weighted means (loess).

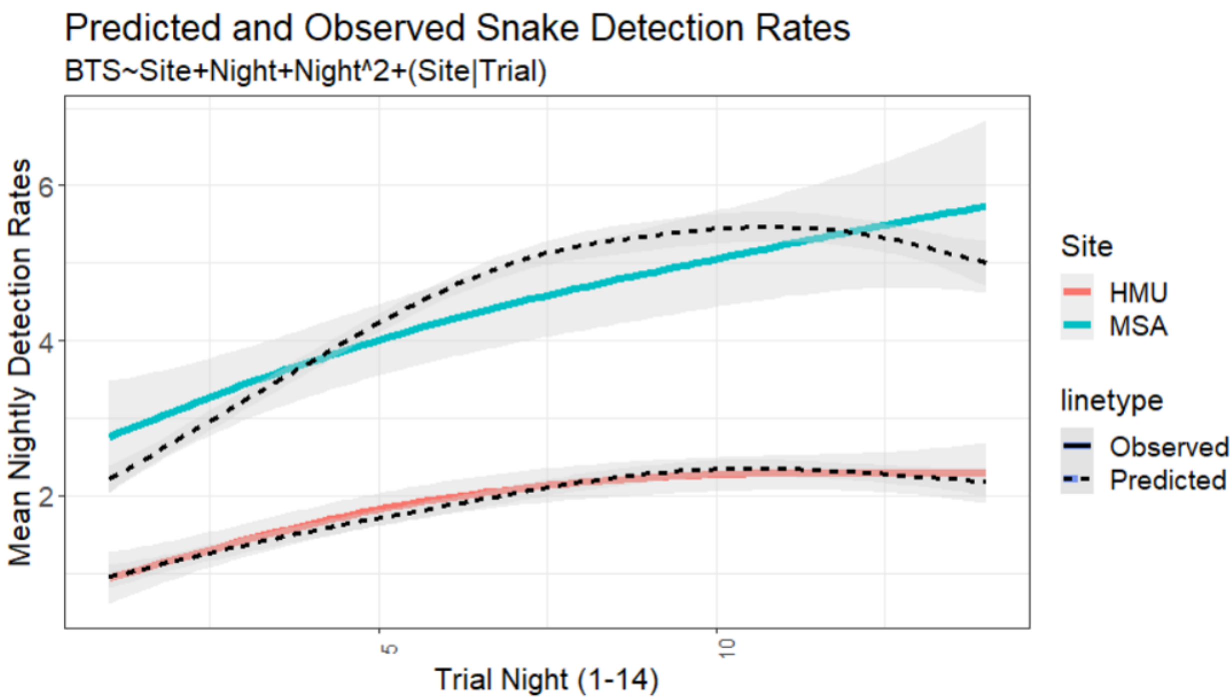
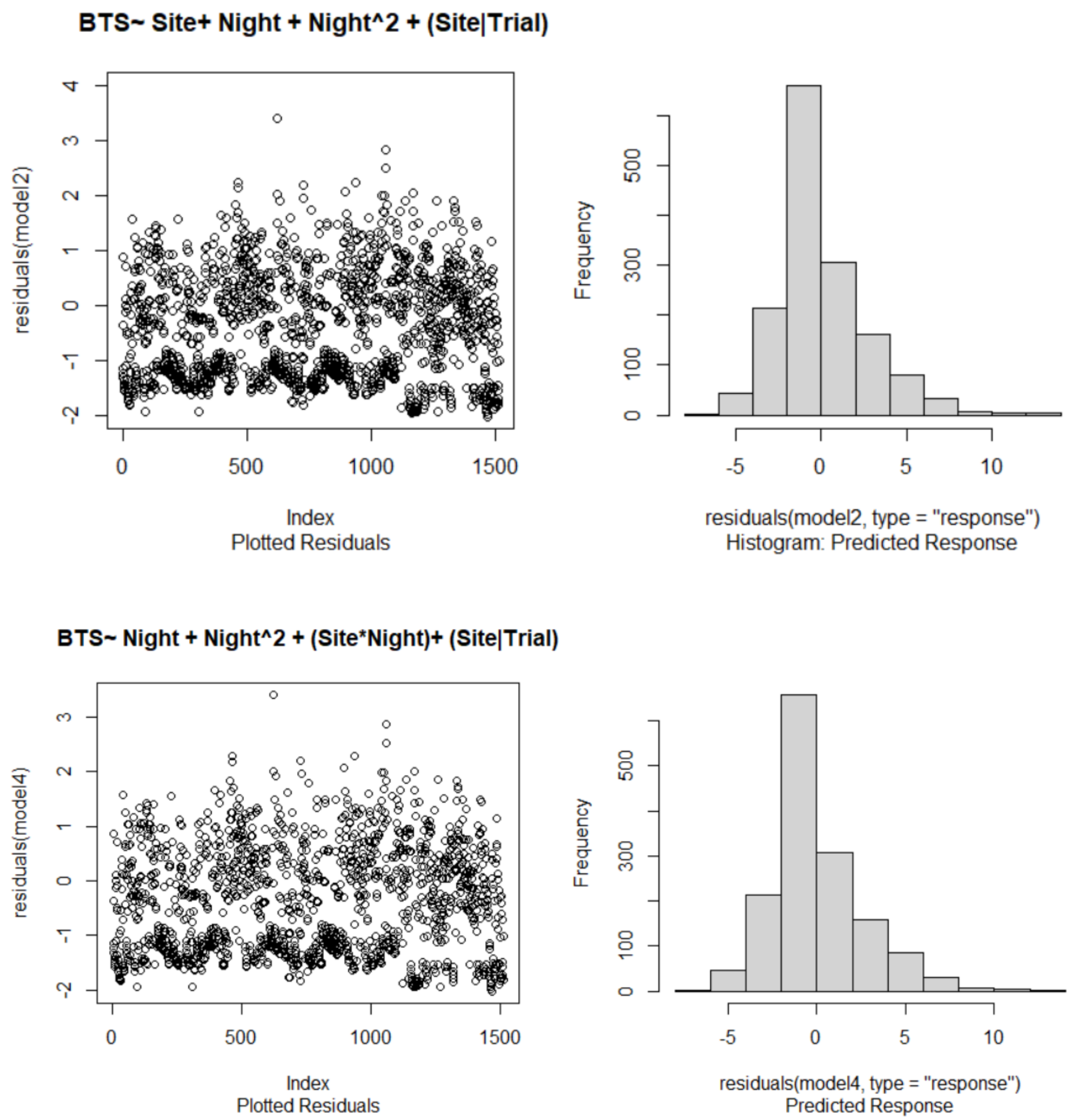
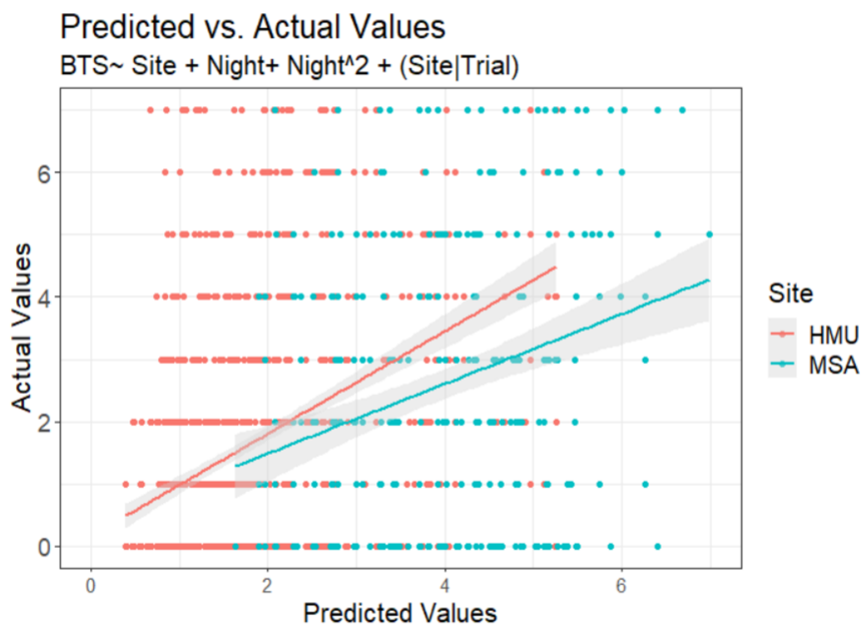


Figure 1d) We plotted the predicted and observed mean snake detection rates during a 14-night trial. The solid lines indicate observed data for the HMU (red) and the MSA (blue). Dashed lines indicate predictions from Model 2 (Table 1b). The solid lines indicate observed data for the HMU (red) and the MSA (blue). Terms for Study Site, Night, and Night^2 were all significant (P<.01). Predictions were applied separately to either study site. Grey shaded areas indicate confidence intervals (CI=0.95).



Figures 1e) We modeled snake detection rates in relation to the study site and trial night (nights 1-14) across all data. We plotted the residual plots for our negative binomial models. *(Top)* Model terms include fixed effects for *Night and Night^2* and the random effect *(Site|Trial*) (Model 1, Table 1b). (*Below)* Model terms also include an interactive term (Site*Night), which did not have a significant effect ($P > .05$), (Model 2, Table 1b). The fitted residuals illustrate that

a majority of the residual values lie between y= -2 and y= 2, indicating a typical distribution pattern for a negative binomial regression; (*Left*) Points indicate the plotted residuals; (*Right)* a Histogram of residual response predictions.



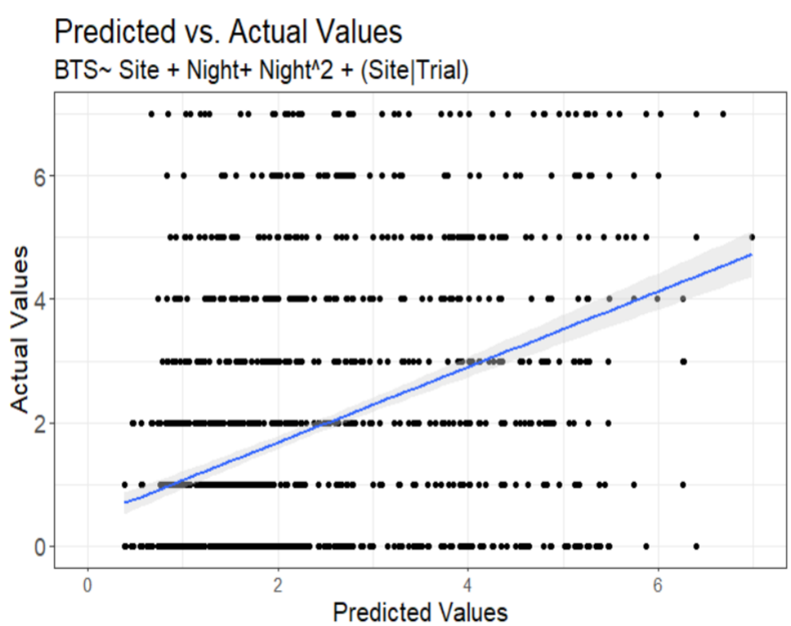
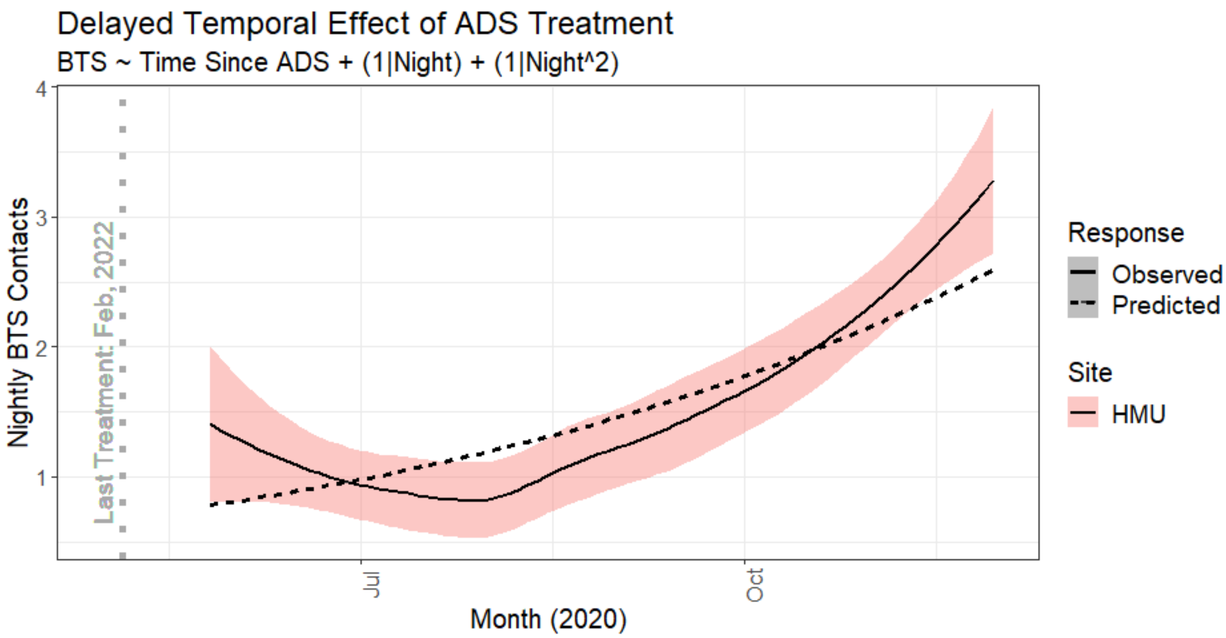


Figure 1f) We plotted predicted (y-axis) nightly snake detection rates against observed (x-axis) snake detection rates. Predictions are based on a negative-binomial model with Site, Trial Night and Night^2 as fixed effects (Table 1b). The solid lines indicate the linear relationship between predicted and observed data; shaded areas represent the margin of error (CI=.95); points indicate nightly count data. *(Above) We applied* predictions separately to the treated (red) and untreated (blue) study sites (above) to compare the model fit for either study site; less data was collected in the MSA leading to a wider margin of error. (*Below)* We applied model predictions to all data.



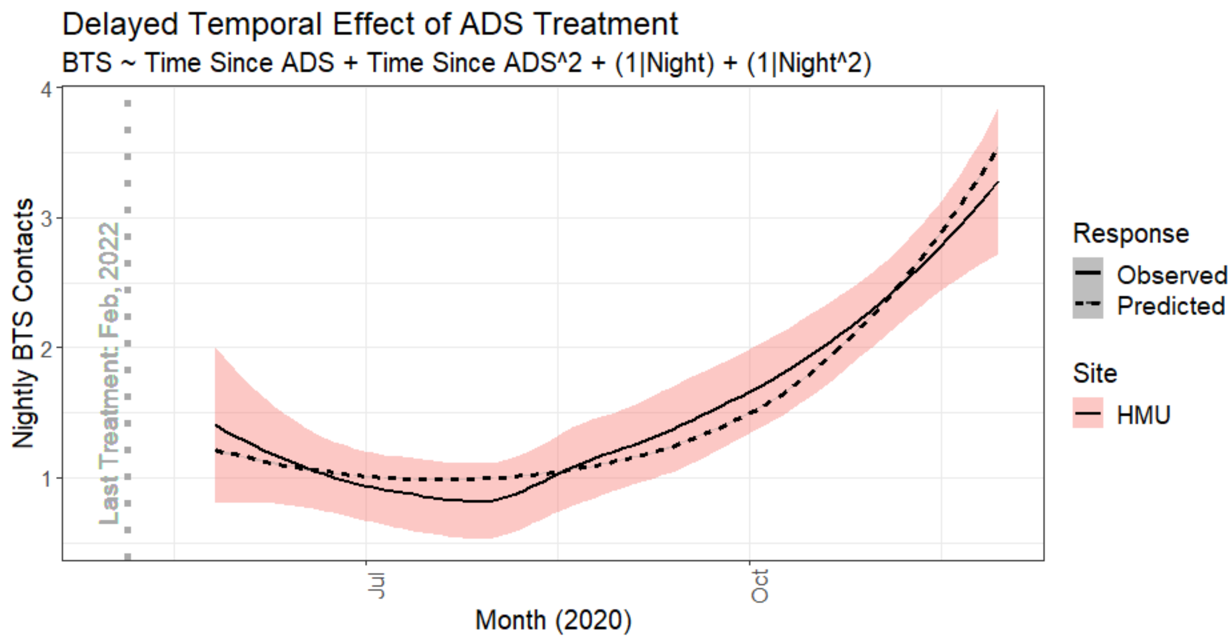


Figure 2a) We plotted model predictions (dashed line) and observed data (solid line) to observe temporal trends in snake detection rates observed by camera platform monitoring. The solid black lines indicate observed data, and the dashed line indicates model predictions (Table 2a, Model 2). The shaded area indicates the confidence intervals (CI=0.95). We modeled detection rates in relation to increasing time since the last treatment application and also included a quadratic term for time since treatment (below); the quadratic term improved the fit of the model compared to a model without the quadratic term (above). We found that detection rates significantly increased with time (P (CDate) < .01, β = -2.699, SE=.819; P (CDate^2) < .01, β = .0001, SE=.00002). The red dotted line indicates the last ADS treatment (February 2020) to occur before camera-platform monitoring began (April 2020). Note that the first ADS treatment occurred in February of 2020, but the line was placed later on the x-axis to improve the visual scale of the plot.

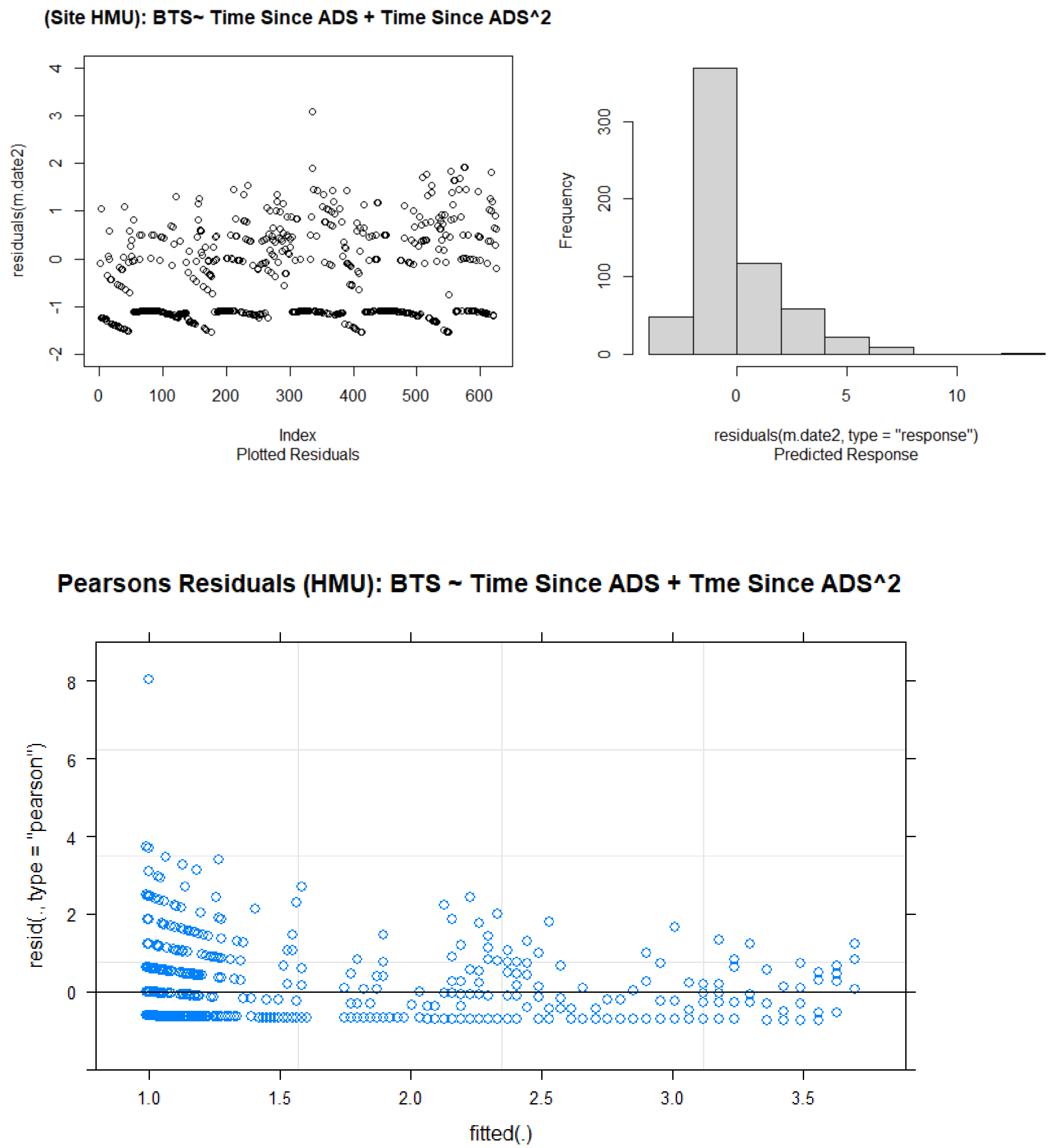


Figure 2b) We modeled the relationship between detection rates and time passed since ADS treatment (continuous and quadratic) in the HMU, and we plotted the residuals. *(Below)* When plotted, Pearson's residuals illustrate that a majority of the residual values lie between y= -2 and y= 2, indicating a typical distribution pattern for a negative binomial regression; (*Top Left*)

Points indicate the plotted residuals, which lie mostly between Y=2 and y=-2; (*Top Right)* a Histogram of residual response predictions.

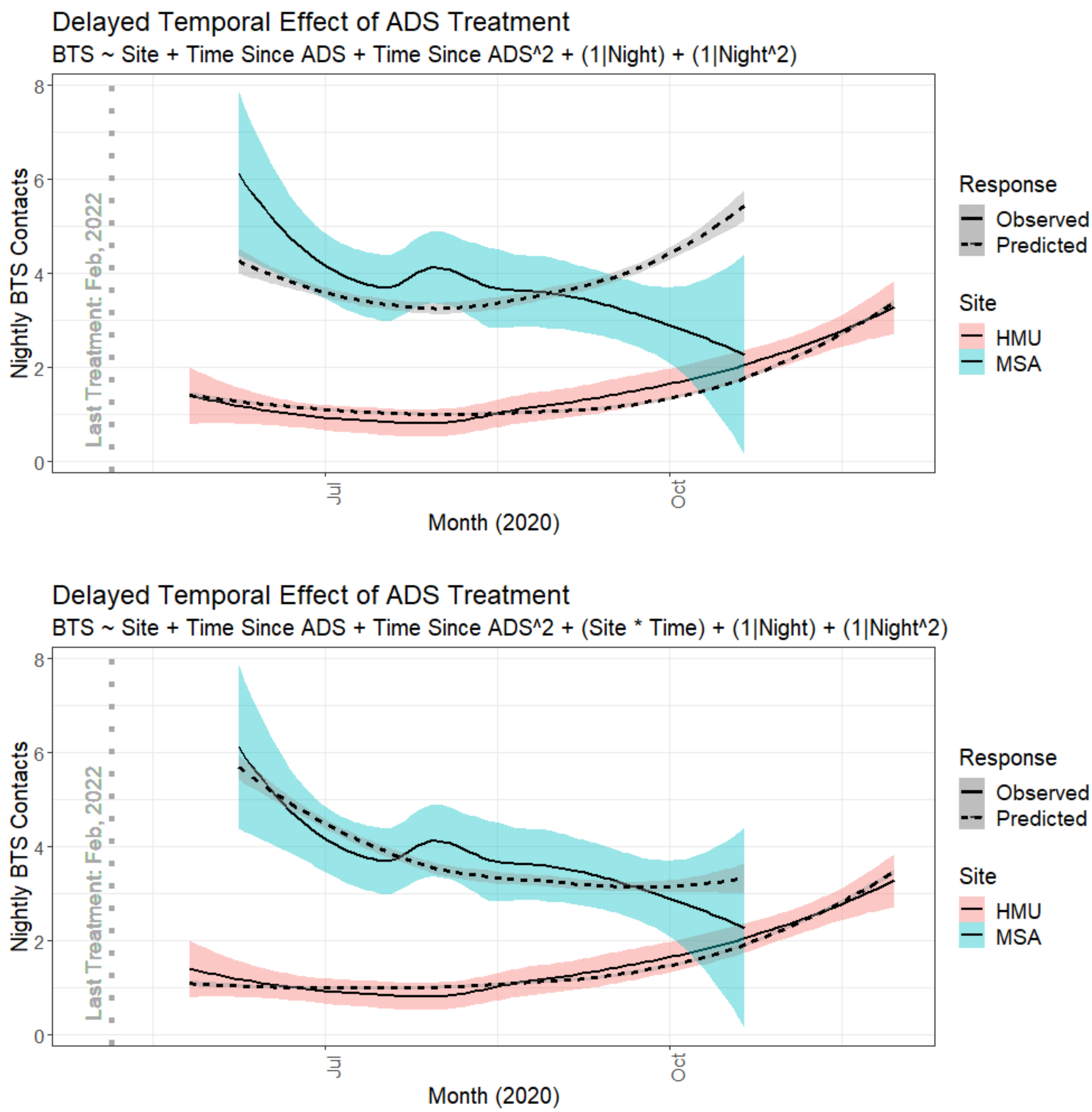
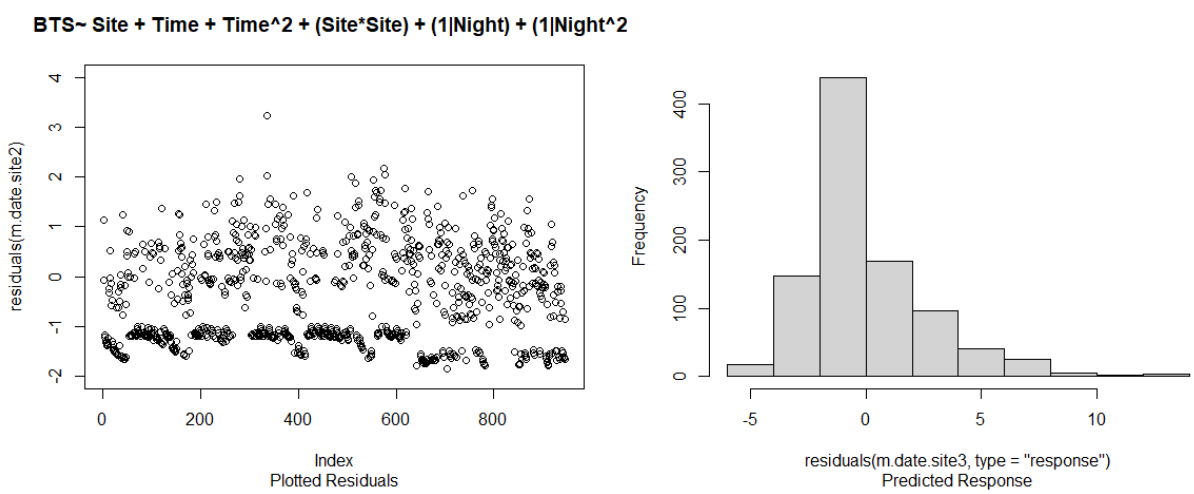


Figure 2c) We compared temporal trends in snake detection rates by study site comparison during a ~9-month period when aerial treatments halted in the HMU. We modeled detection

rates in relation to the amount of time since aerial treatment and study site, with interaction between site and time to allow trends to vary differently in treated and untreated areas. When we included an interactive term, model predictions were better fit to observed data *(below)* compared to a non-interactive model *(above)*. We found that the expected detection rates in the HMU increased significantly with increasing time since the last treatment application (in February 2020). The expected detection rates in the HMU increased by roughly 150% since the start of the monitoring period to the start of November; in the MSA detection rates decreased by 67%.



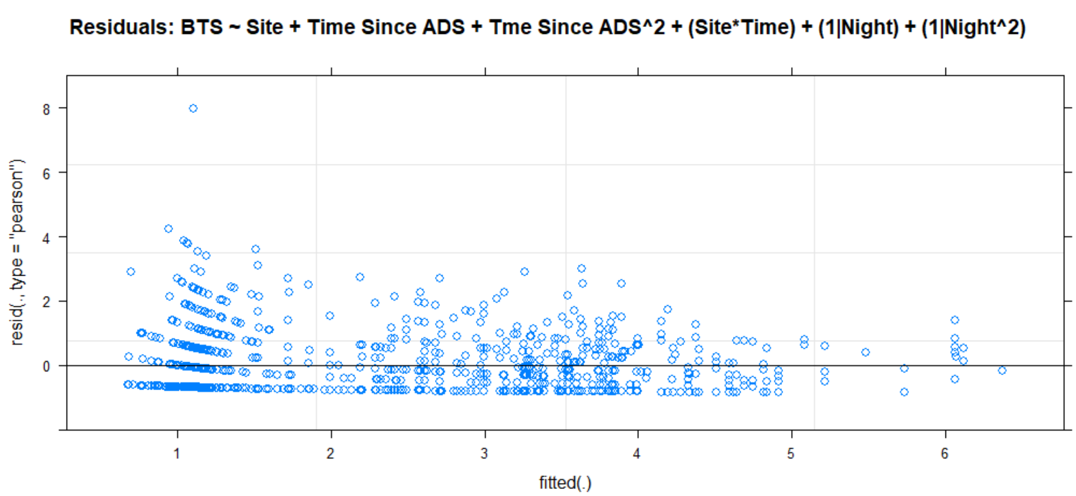


Figure 2d) We modeled the relationship between detection rates and time passed since ADS treatment (continuous and quadratic) with an interactive term for study sites. *(Below)* When plotted, Pearson's residuals illustrate that a majority of the residual values lie between y= -2 and y= 2, indicating a typical distribution pattern for a negative binomial regression; (*Top Left*) Points indicate the plotted residuals, which lie mostly between Y=2 and y=-2; (*Top Right)* a Histogram of residual response predictions.

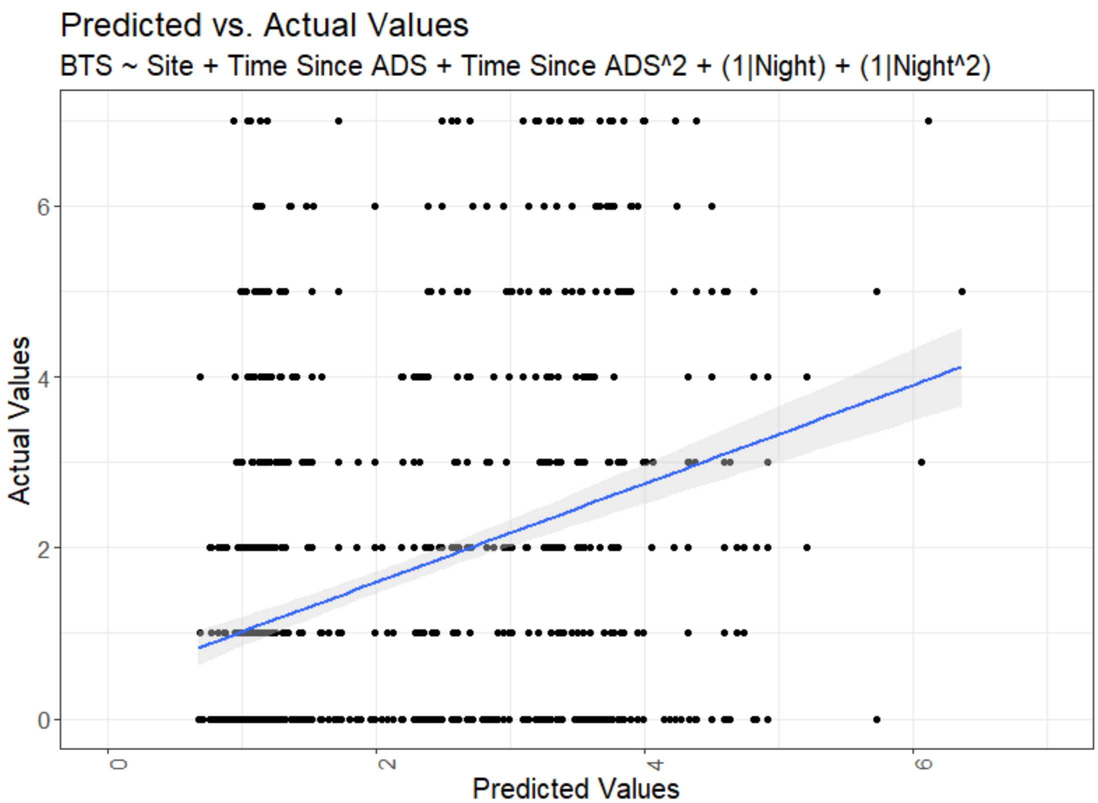


Figure 2e) We plotted the predicted nightly detection rates (x) against observed nightly detection rates (y). *(Above)*: We grouped data by study site to evaluate difference in the linear relationship for data from the HMU (red) and the MSA (blue). *(Below)*: We evaluated all data regardless of study site. Points indicate nightly detection rates, and lines indicate the linear relationship between predicted and observed values. Shaded areas indicate confidence intervals (CI=0.95). Model predictions were better fitted in HMU data than the MSA, likely because there was more data from the HMU.

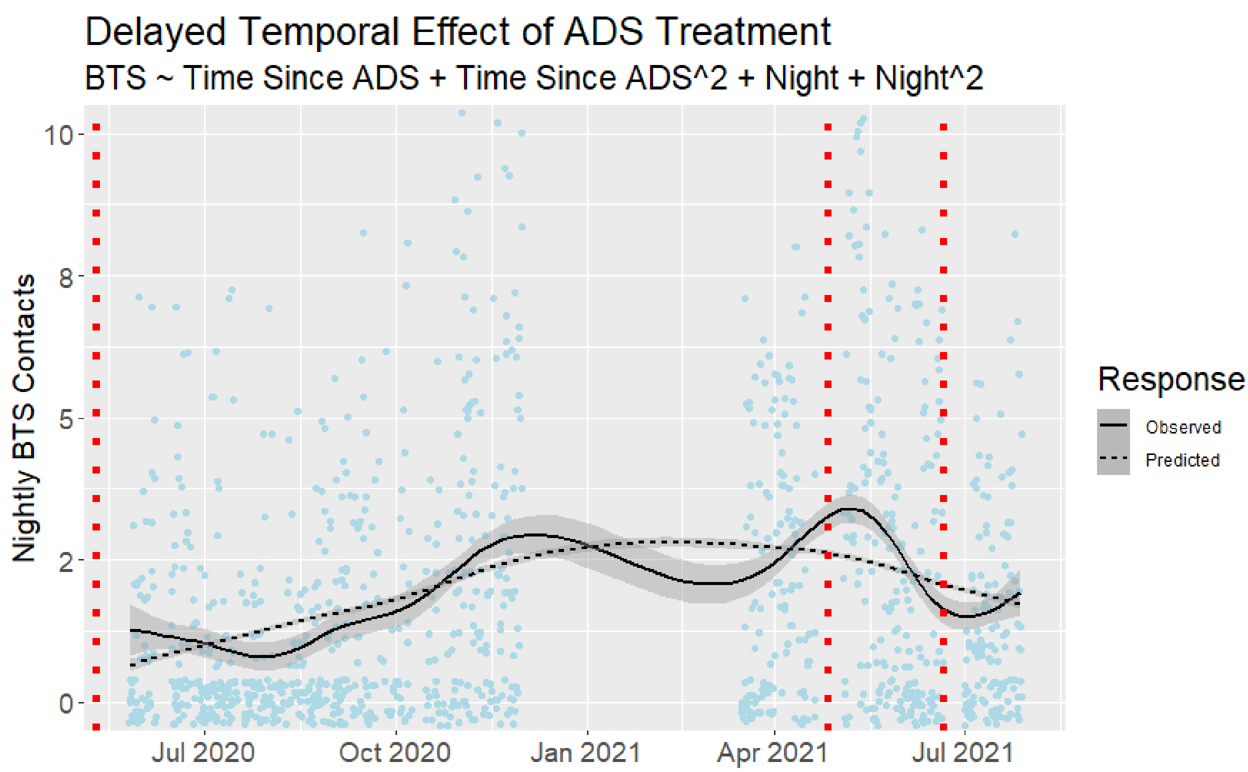


Figure 2f) We experimentally applied model predictions for expected snake detection rates to the extent of data collected in the HMU (Table 2a, Model2). Red dotted lines indicate ADS treatments. ADS treatments occurred in February of 2020, and in April and May of 2021. Predicted snake detection rates are indicated by the dashed line. The shaded area indicates the confidence intervals (CI=.80). Light blue points indicate observed nightly contact rates, and the solid line indicates trends in mean detection rates over time using locally weighted averages (loess), which can also be applied to forecast non-linear trends. However, there are sizeable gaps in this data that may be misleading with the use of the smoothing method. We found that model predictions demonstrate both the recovery and the suppression effects that align with application dates of aerial treatment. In this model, we used non-quadratic and quadratic terms for time passed since ADS but included more data than before. Both terms were significant (P < .01). *Note that the line indicating the first ADS treatment (February of 2020) was placed at a later date on the x-axis to improve the visual scale of the plot.

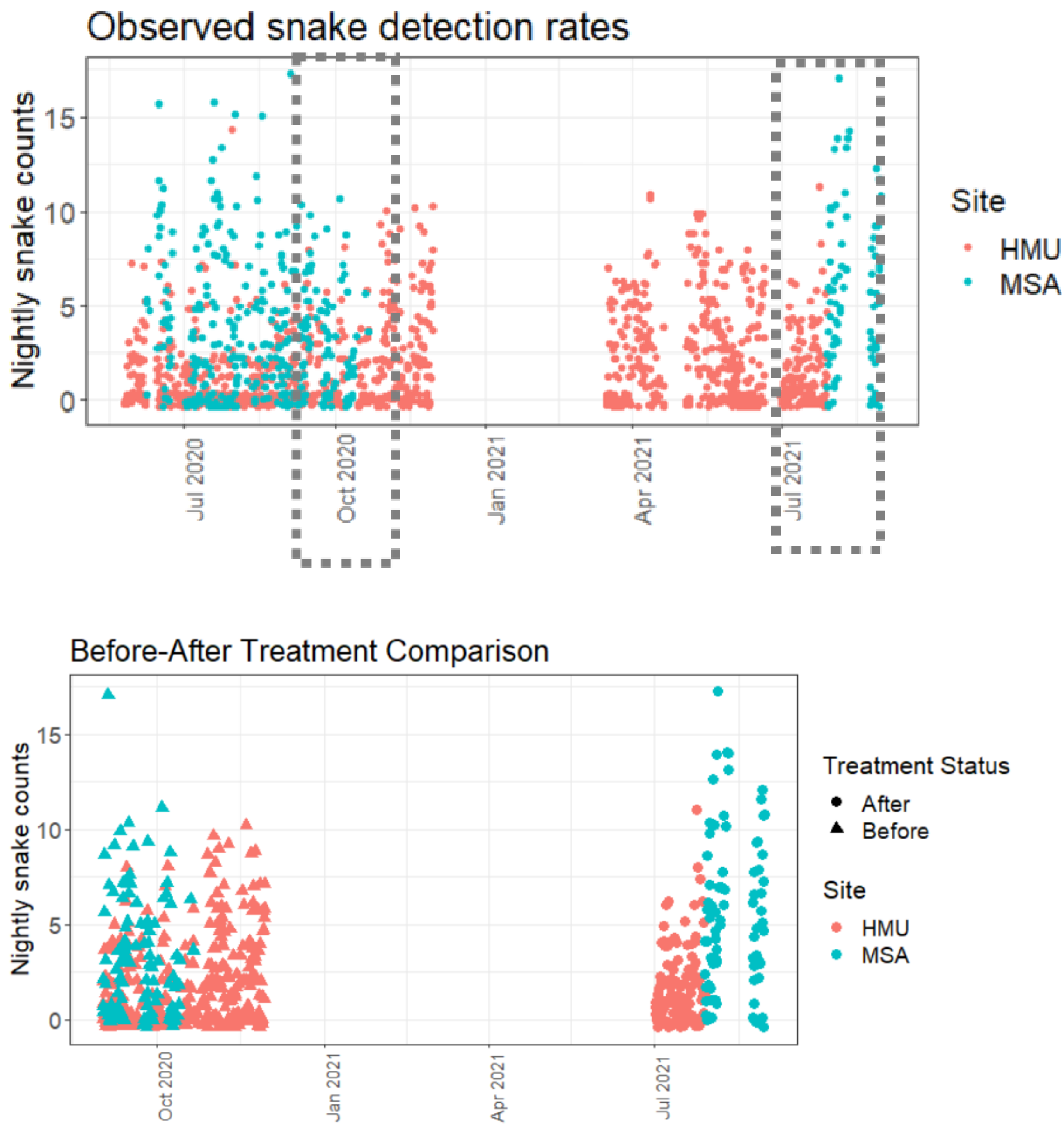


Figure 3a) We evaluated data from both study sites (HMU=red, MSA=blue) to compare snake detection rates before and after treatments. The amount of data for time periods when both study sites were monitored was limited, but data for both sites was defined within the same two-month period before or after treatment. *(Above)* We plotted the entire timeline of data collected, with grey dashed boxes to illustrate the subsets of data to represent snake detection rates in relation to

treatments. *(Below)* We plotted the nightly detection count data (points) included in the before and after treatment comparison (Triangle=Before, Point=After).

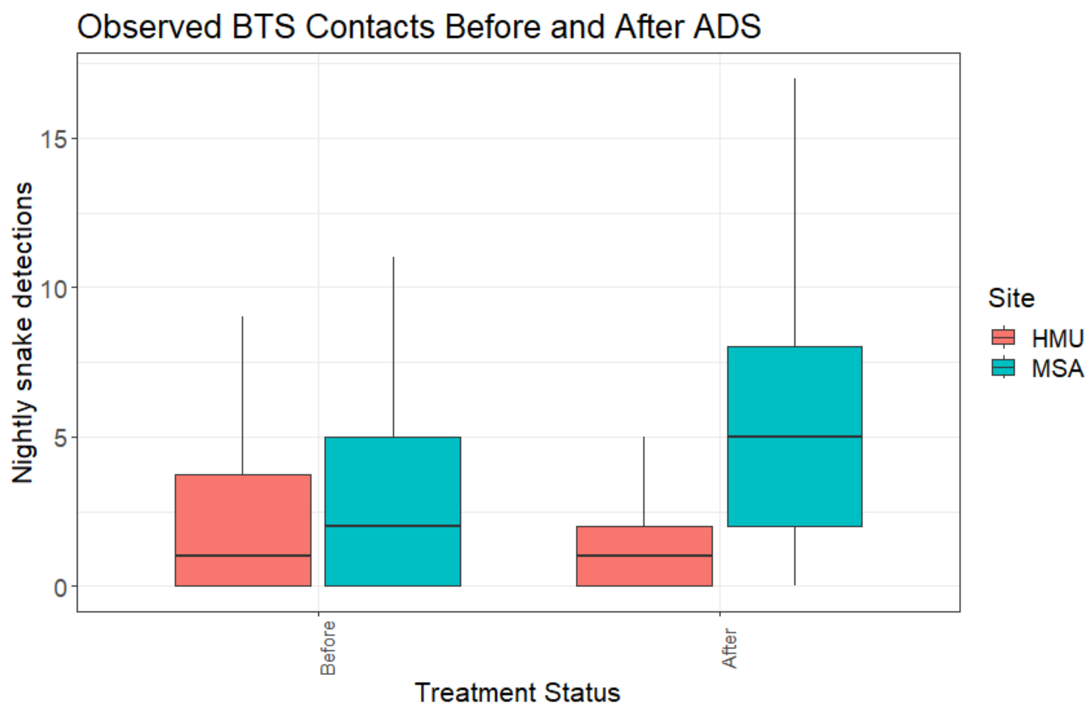


Figure 3b) We evaluated data from both study sites (HMU=red, MSA=blue) to compare snake detection rates before and after treatments. We created a boxplot of observed nightly snake detection rates (y-axis) before and after aerial treatment (x-axis). Observed mean snake detection rates in the HMU were 2 (SD=3) before and 2 (SD=2) after ADS. In the MSA they were 3 (SD=3) before and 5 (SD=4) after ADS (Figure 3b).

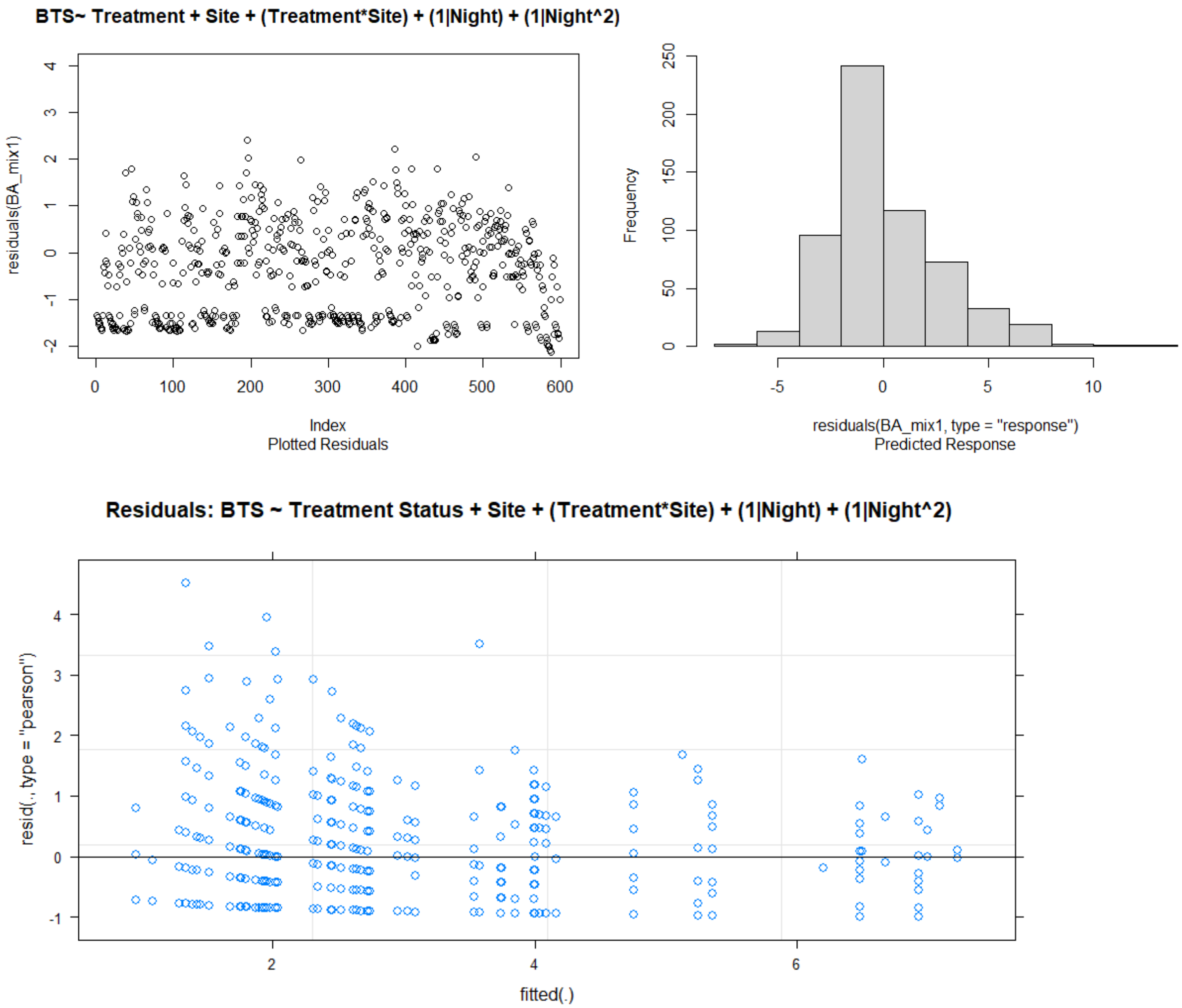


Figure 3c) We modeled snake detection rates in relation to the study site and treatment status (before or after ADS treatment), and we plotted the residual plots for our negative binomial models. *(Below)* When plotted, Pearson's residuals illustrate that a majority of the residual values lie between y= -2 and y= 2, indicating a typical distribution pattern for a negative binomial regression; (*Top Left*) Points indicate the plotted residuals, which lie mostly between Y=2 and y=-2; *(Top Right)* a Histogram of residual response predictions.

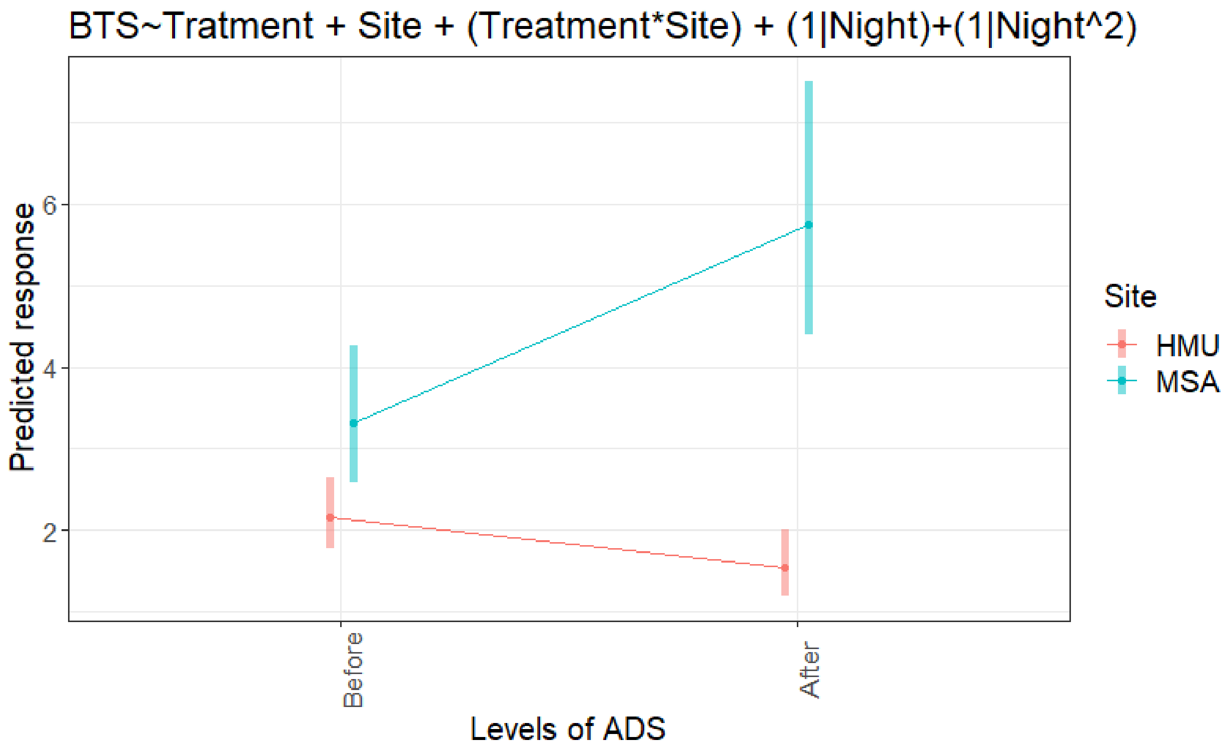
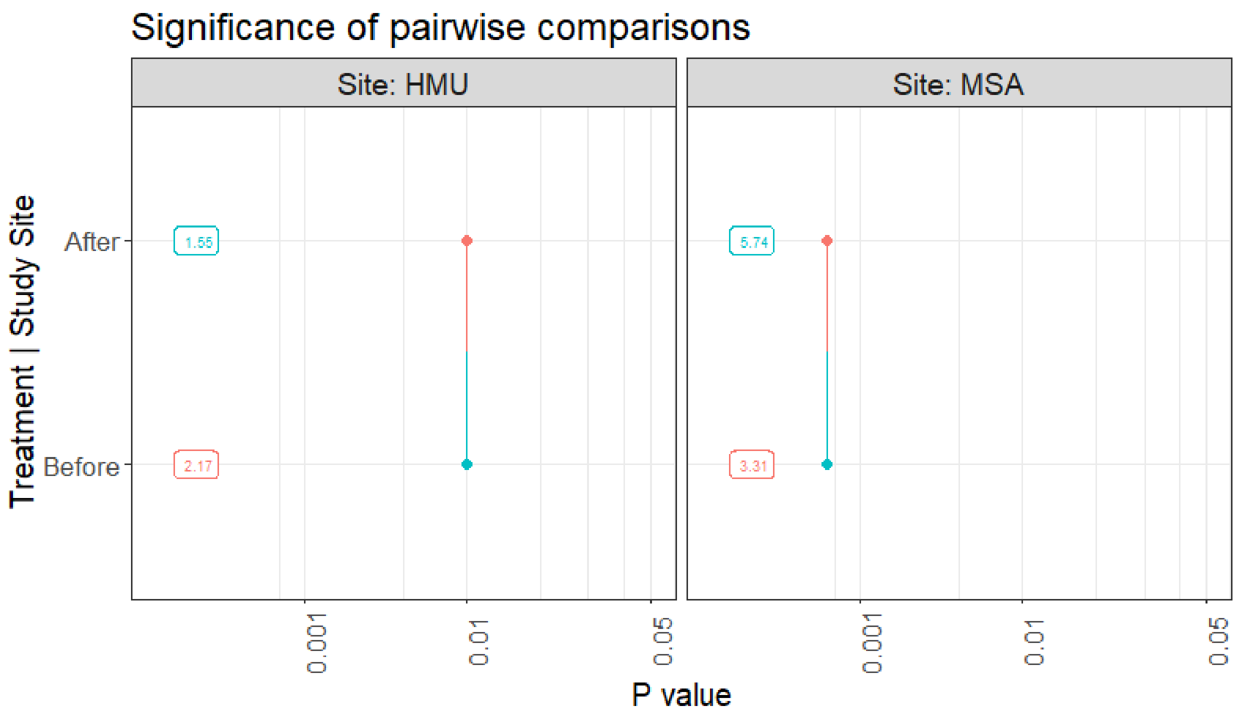


Figure 3d): We plotted an effects plot of model predictions for snake detection rates in the HMU (red) and the MSA (blue) before and after treatment. The MSA did not receive treatment but was used as a reference study site as a reference to changes in relative snake abundance during the same "Before-After" time period in the absence of treatment.). In the HMU, we found that the expected mean snake detection rates were 2.17 before and 1.55 after treatment. In the MSA, the expected mean detection rates were 3.31 before and 5.74 after treatment. The shaded vertical lines represent error bars (CI=0.95). The solid lines indicate the treatment effect for either study sites. The space between CI lines indicates the significance level of the comparison, and overlapping error bars implies lack of statistical significance (P>.05). Predictions were based on a mixed-effects model (Table 3a) with pairwise comparisons performed to evaluate all combinations of factors levels using the *emmeans* function in R (Table 3b). Fixed effects

included interactive terms for Study Site and Treatment Status; random effects included continuous and quadratic terms for Trial Night, and a term for the Trial nested by Study Site.



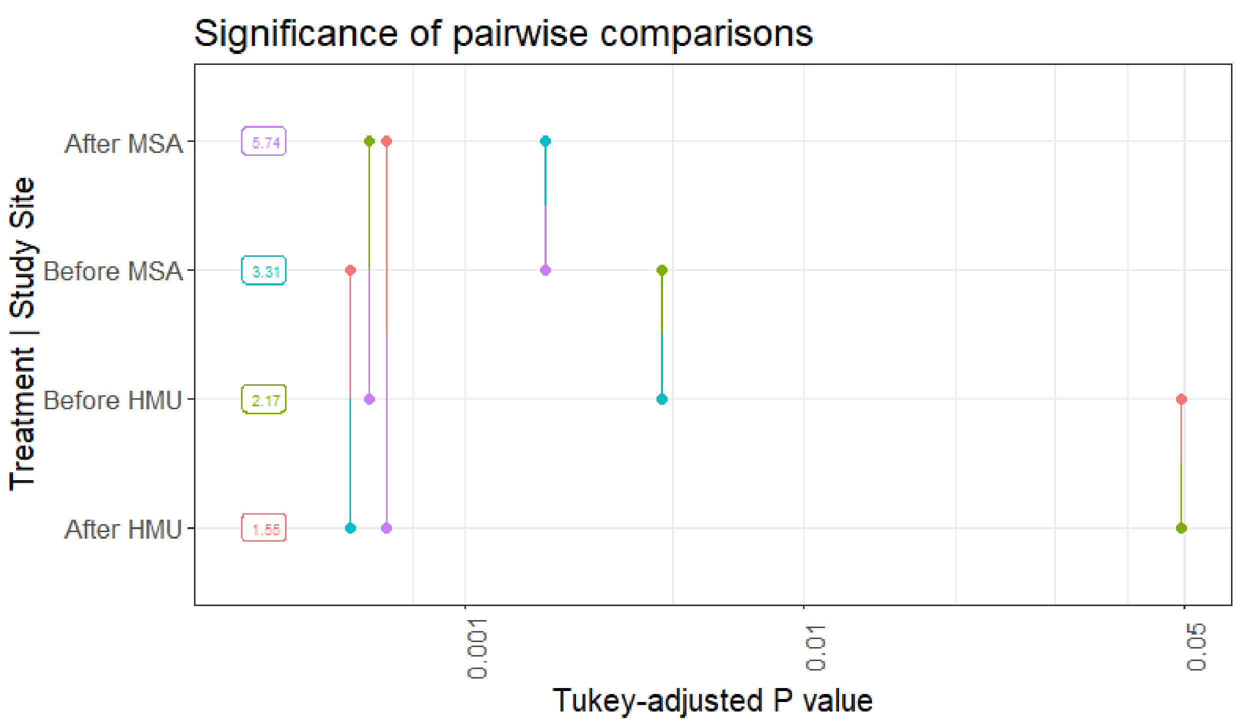
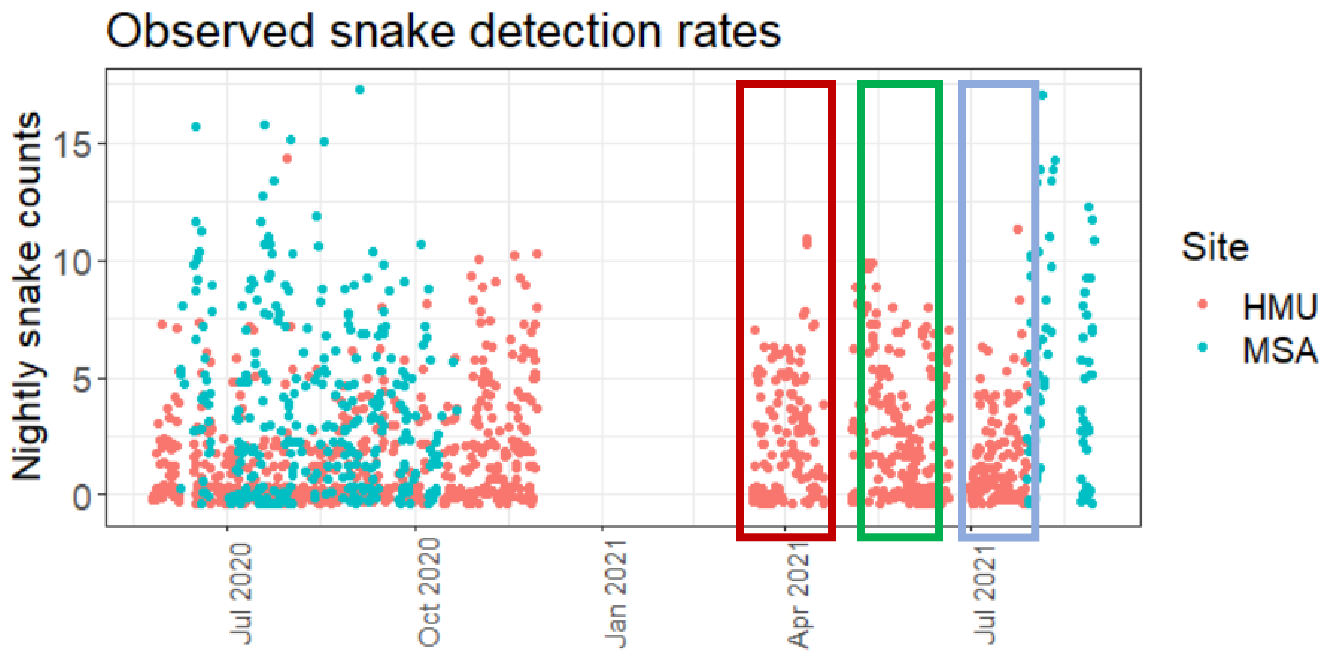


Figure 3e) We plotted significance levels for *emmeans* comparisons of snake detection rates by Study Sites (HMU and MSA) and treatment status (before or after) (y-axis) are plotted according to the p-value associated for each comparison. The estimated mean snake detection rate for category of variables is printed to the right of corresponding y-axis labels. We included interactions between factor levels produced the same model predictions (Below) and plotted pairwise comparisons applied to the same model without interactions for every possible factor level (Above). We performed pairwise comparisons using the *emmeans* function in R (table 3b).



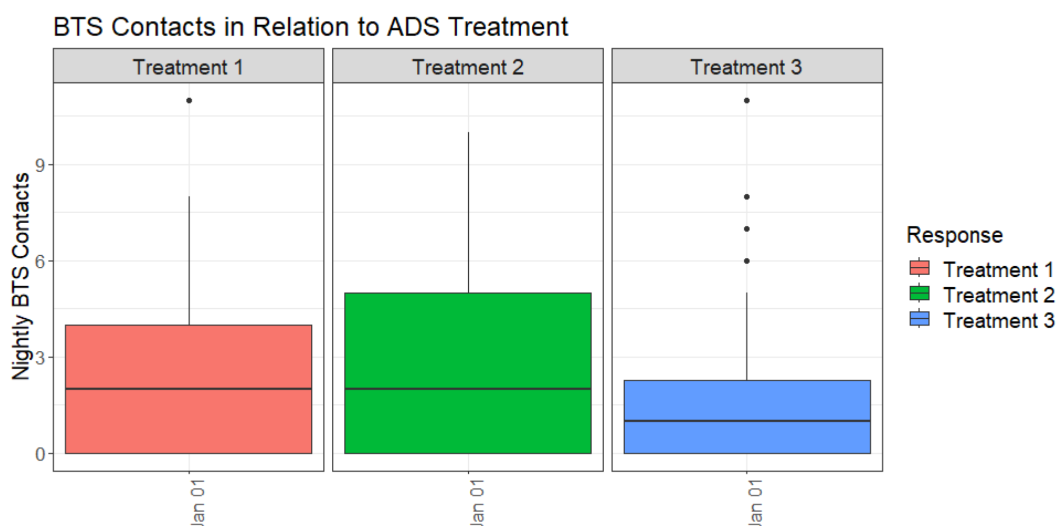


Figure 3f) We evaluated snake detection rates across three treatment periods that occurred before, between and after successive aerial treatments. We found that the observed mean nightly snake detection rates were 2.5 during treatment 1, 2.8 during treatment 2, and 1.7 during treatment 3. *(Above)* We plotted snake count data across the extend of the dataset and placed boxes around the treatment periods. *(Below)* We plotted nightly snake contact rates during

repeated ADS treatments. Points indicate observed nightly snake counts with color corresponding to the boxes that outline treatment periods in the timeline above (1=red, 2=green, 3=blue). The trend lines indicate temporal trend in mean nightly contacts using local weighted means of detection rates. Grey shaded areas indicate confidence intervals (CI=.95).

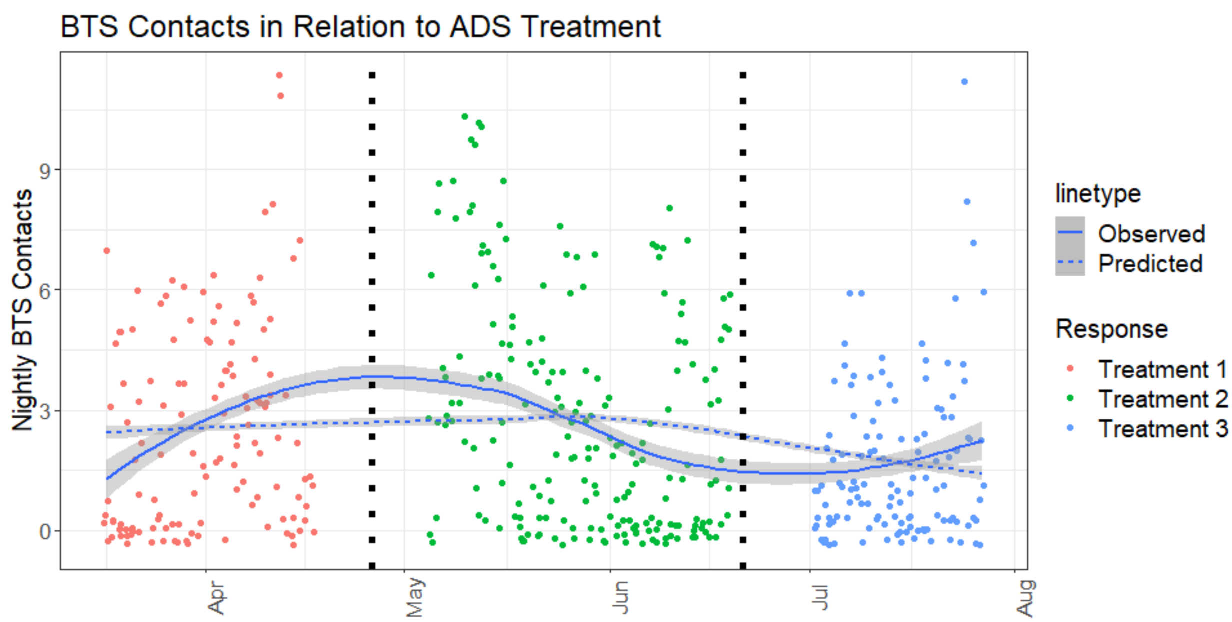
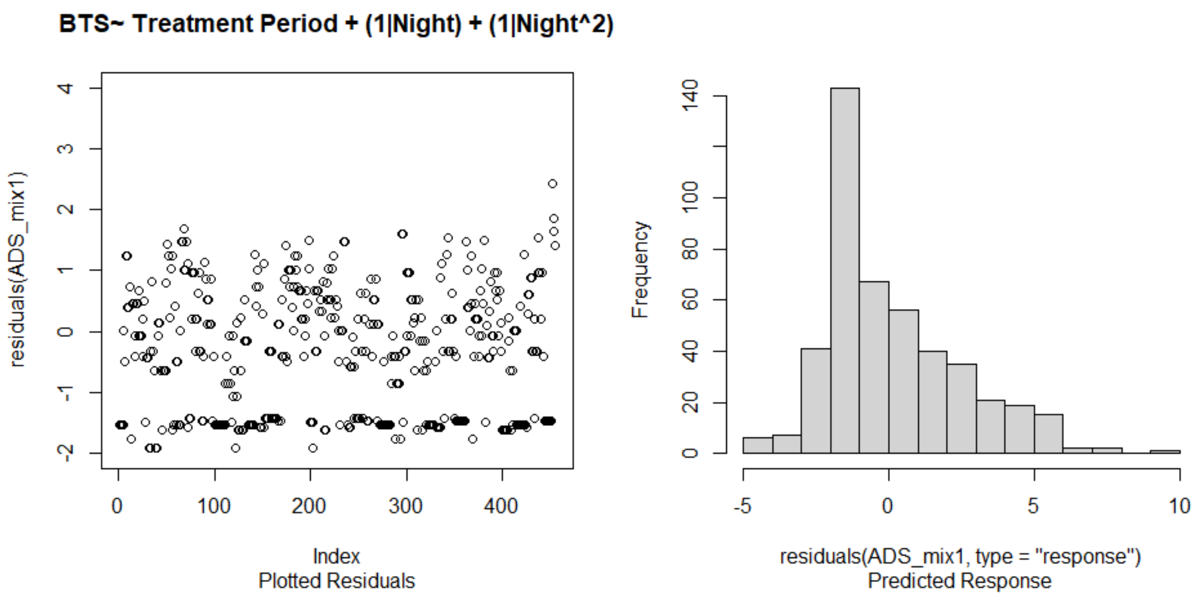


Figure 3g) We modeled detection rates in the HMU in relation to the Treatment Period: 1) Before treatments, 2) Between two treatments, and 3) after two treatments. Points indicate nightly snake detection count data, and their colors indicate the Treatment Period. Trend lines indicate mean temporal trends in observed (solid) and predicted (dashed) detection rates. We included random effects for trial night and Night^2, which accounted for temporal variability that was not related to treatment effects, allowing model predictions to display treatment effects by treatment period.



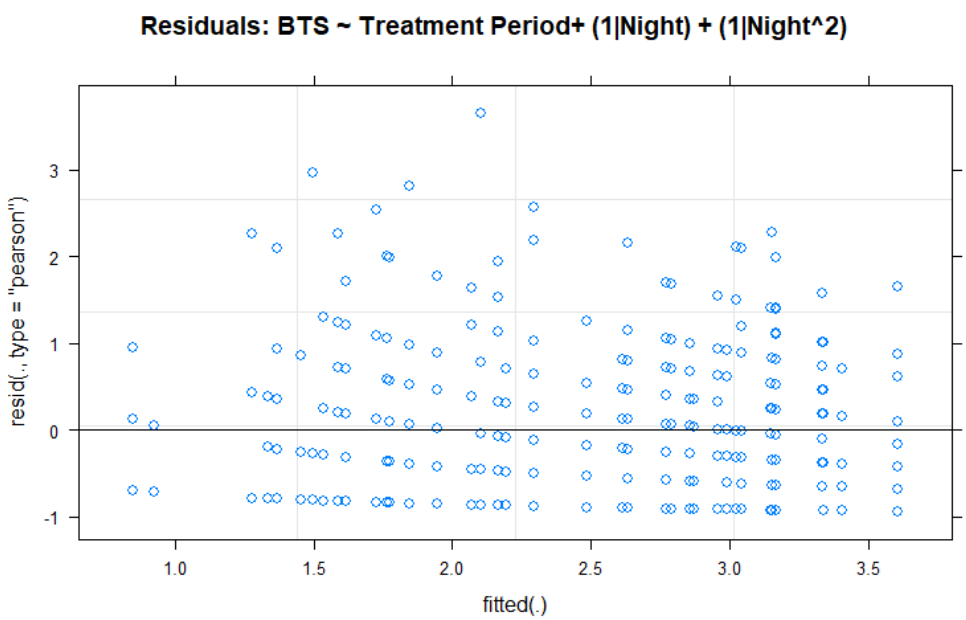


Figure 3h) We modeled snake detection rates in relation to the Treatment Period: 1) Before treatments, 2) Between two treatments, and 3) after two treatments and plotted the residuals; *(Below)* When plotted, Pearson's residuals illustrate that a majority of the residual values lie between y= -2 and y= 2, indicating a typical distribution pattern for a negative binomial

regression; (*Top Left*) Points indicate the plotted residuals, which lie mostly between Y=2 and y=-2; (*Top Right)* a Histogram of residual response predictions.

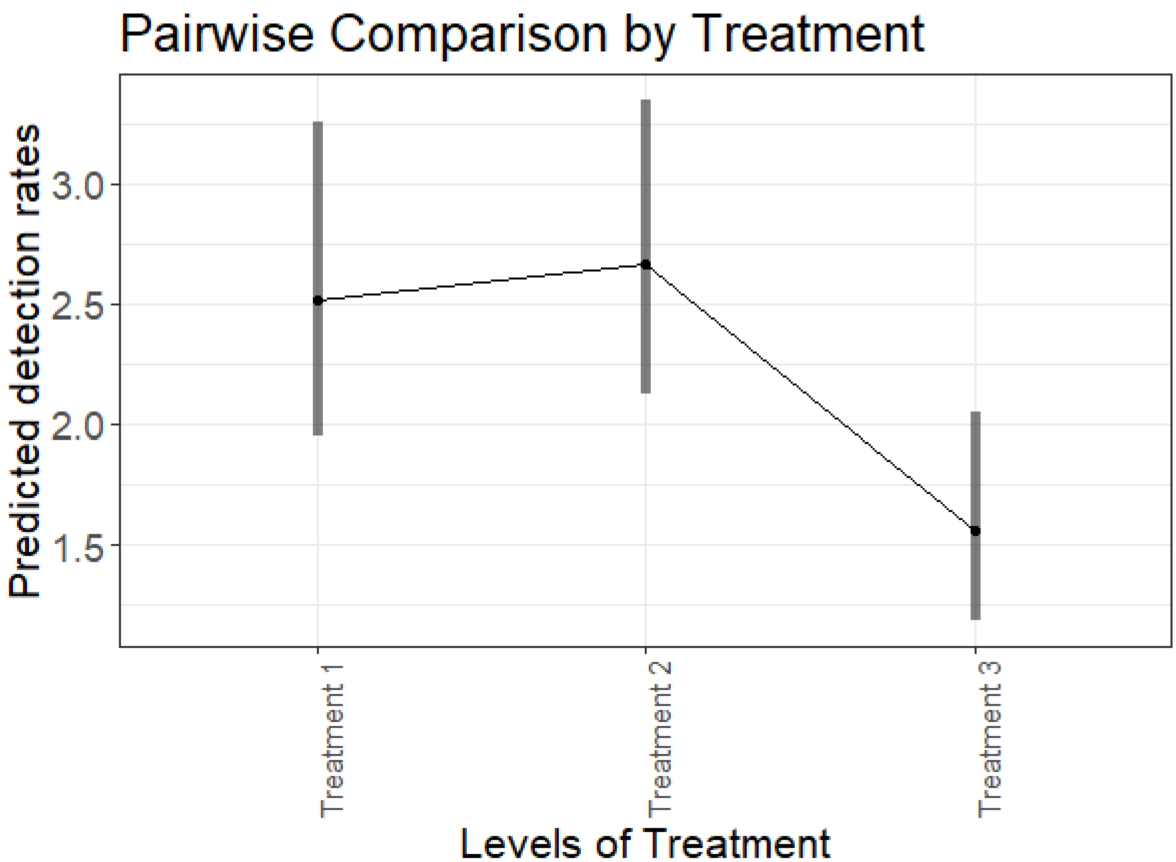


Figure 3i) We plotted model predictions based on pairwise comparisons applied to a mixed-effects model (Table 3a) to evaluate relative change in snake abundance in relation to levels of treatment. We found that snake detection rates did not significantly change from Treatment 11 to 2, but that they decreased significantly during Treatment 3 (P<.01). The solid lines indicate the trend in relative snake abundance across the three treatment periods (before, between, and after the two aerial treatment applications). The shaded vertical lines represent error bars (CI=0.95). We used the *emmeans* function in R (Table 3d).

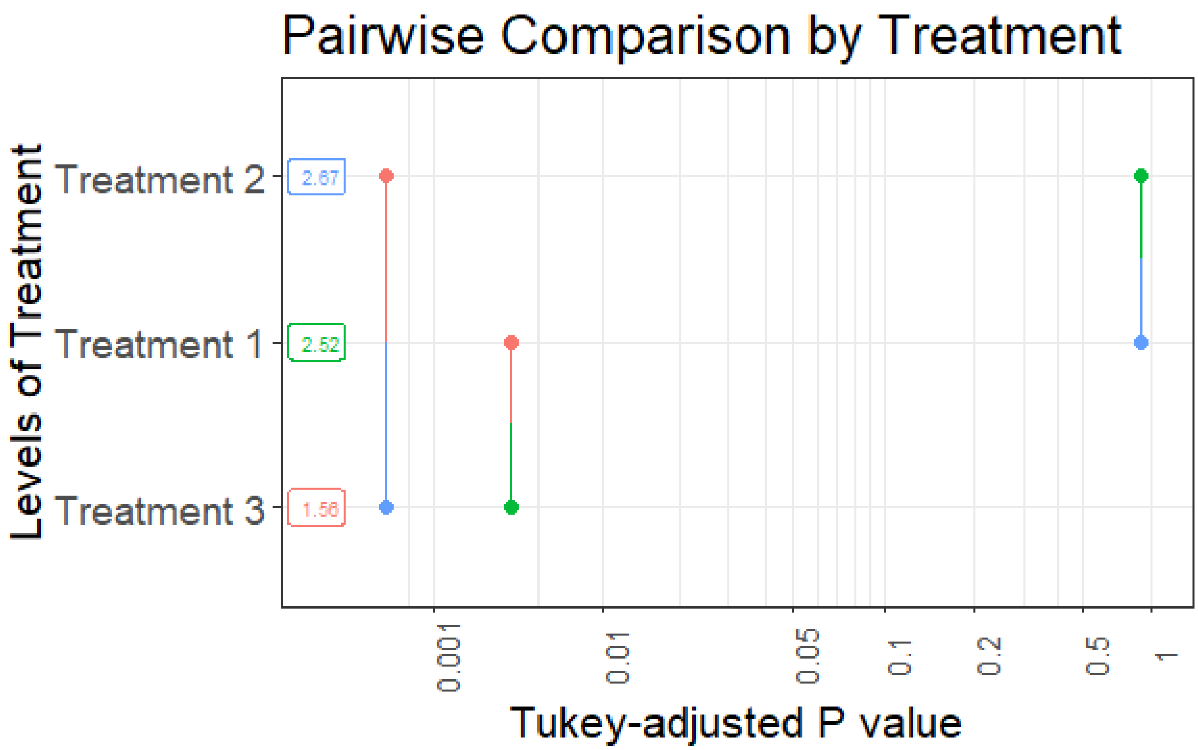
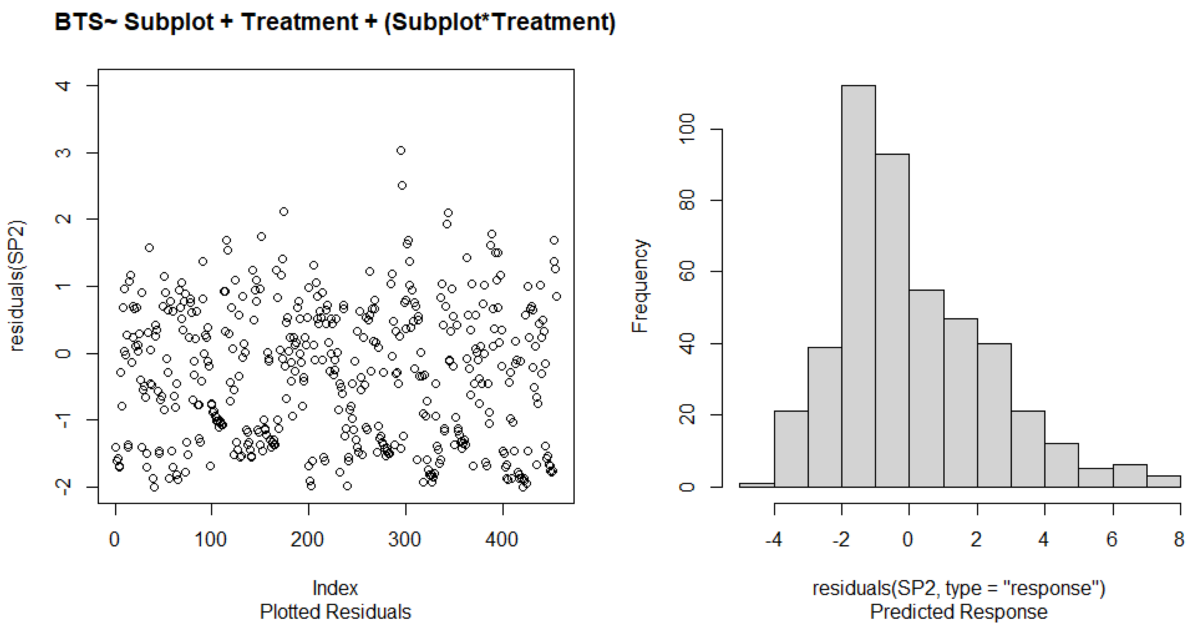


Figure 3j) We performed pairwise comparisons to model predictions of snake detection rates by treatment period in the HMU (table 3b). We compared all factor levels of Treatment period (1= before, 2=between, and 3= after aerial treatments) and plotted their significance levels according to their Tukey-adjusted p-value associated for each comparison (x-axis). The estimated mean snake detection rate for category of variables is printed to the right of corresponding y-axis labels. Interactions among factor levels demonstrated that Treatment 1 and 2 did not significantly vary ($P>0.5$), but Treatment 3 significantly varied from both treatment periods ($P< .01$) in response to two successive aerial treatment applications.



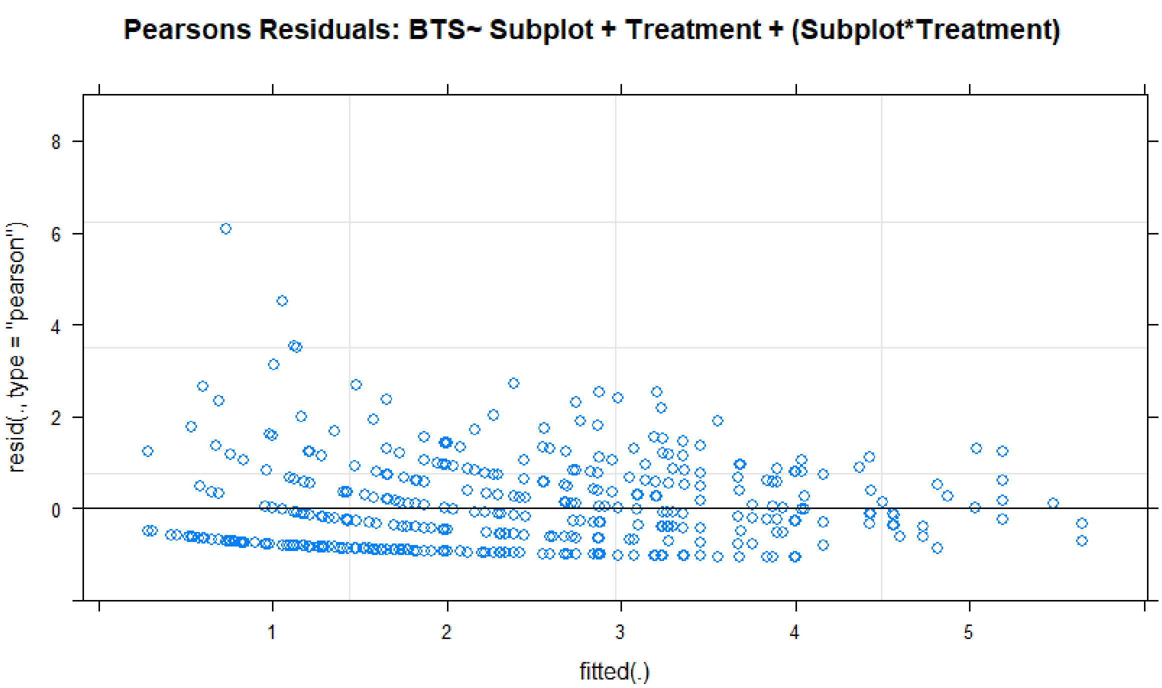


Figure 4a) We modeled snake detection rates in relation to subplot and aerial treatment tatus during three treatment periods. When plotted, Pearson’s residuals illustrate that a majority of the residual values lie between y= -2 and y= 2, indicating a typical distribution pattern for a negative

binomial regression; (*Top Left*) Points indicate the plotted residuals, which lie mostly between Y=2 and y=-2; (*Top Right)* a Histogram of residual response predictions.

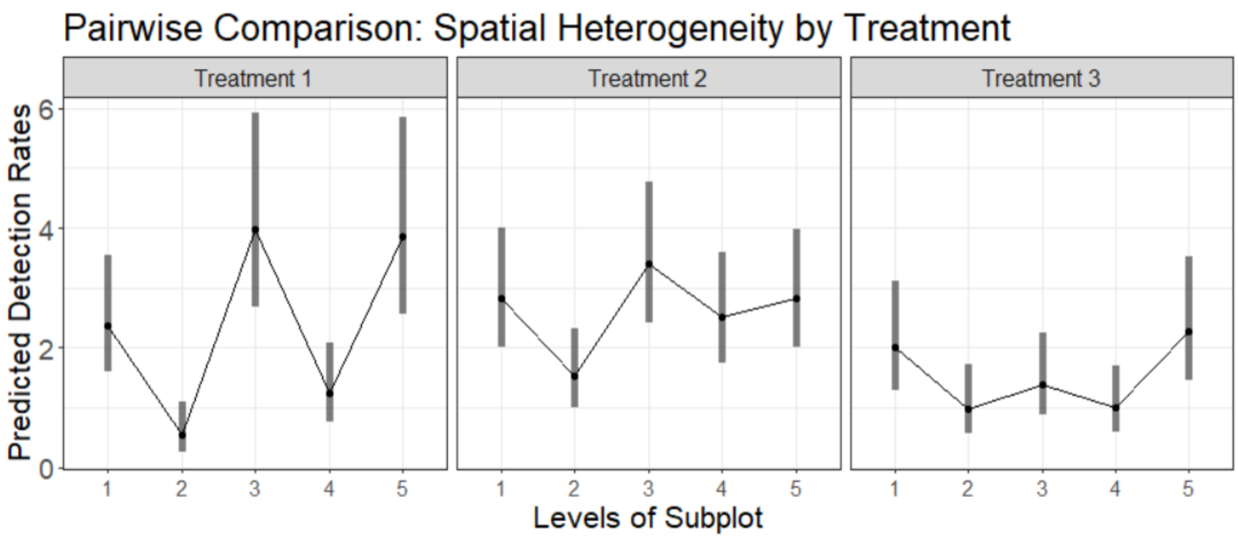


Figure 4b) We compared model predictions for snake detection rates among all subplots in the HMU before, between and after successive aerial treatments. We found that the expected variance among subplots decreased with each treatment period. In treatment period 1 there was significant variance in 5 of 10 subplot comparisons (P<0.01); in treatment period 2 there was significant variance in 1 of 10 subplot comparisons (P<0.05); in treatment period 3, subplot variance was no longer detectable for all subplot comparisons (P>0.05). Predictions were based on a mixed-effects model (Table 4a) with pairwise comparisons performed to evaluate all combinations of factors levels using the *emmeans* function in R (Table 4b). Fixed effects included interactive terms for Subplot and Treatment Status; random effects included continuous and quadratic terms for Trial Night. The shaded vertical lines represent error bars (CI=0.95). The solid lines indicate the treatment effect for either study sites. The space between CI lines indicates the significance level of the comparison, and overlapping error bars implies lack of statistical significance (P>.05).

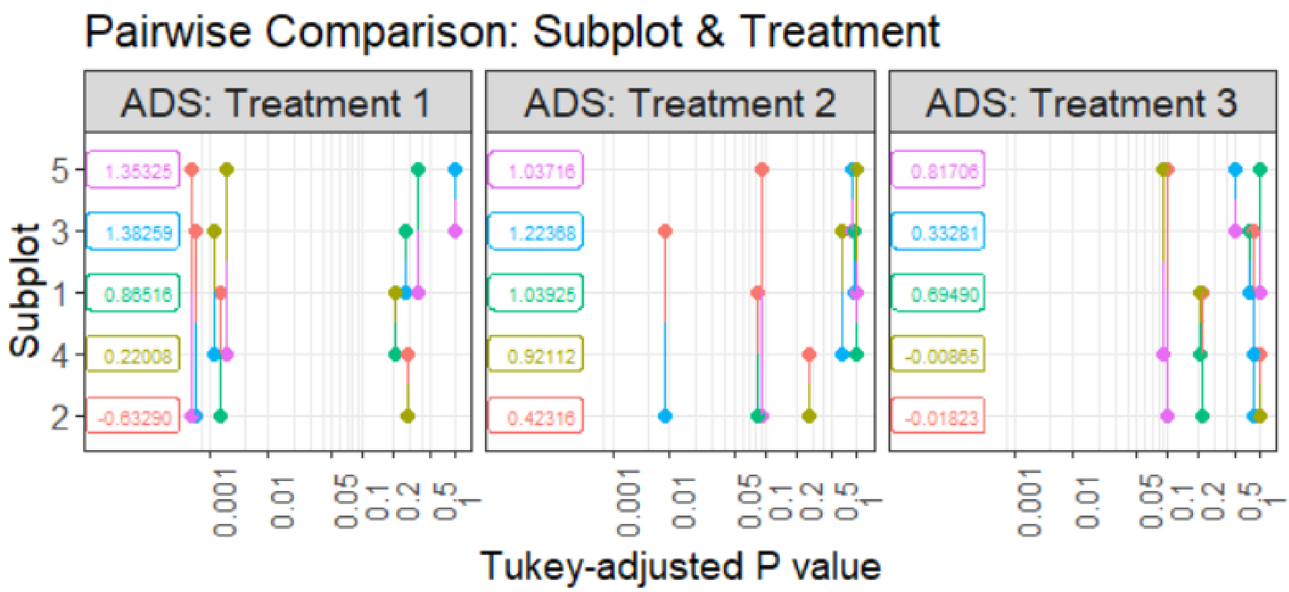


Figure 4c) We plotted the significance levels of pairwise comparisons applied to predicted snake detection rates among subplots before, between and after successive treatments in the HMU (Table 4b). The individual comparisons are demonstrated by the colored bars and are plotted against the p-value associated with their comparison. Subplot comparisons were made for 3 successive Treatment Periods (1= before, 2=between, and 3= after aerial treatments). We found that in treatment period 1 there was highly significant variance in 5 of 10 subplot comparisons (P<0.01); in treatment period 2 there was significant variance in 1 of 10 subplot comparisons (P<0.05); in treatment period 3 subplot variance was no longer detectable among any subplots (P>0.05). The expected nightly snake detection rate is printed to the right of each respective subplot for each treatment period. We based the pairwise comparisons on our mixed-effects model (Table 4a).

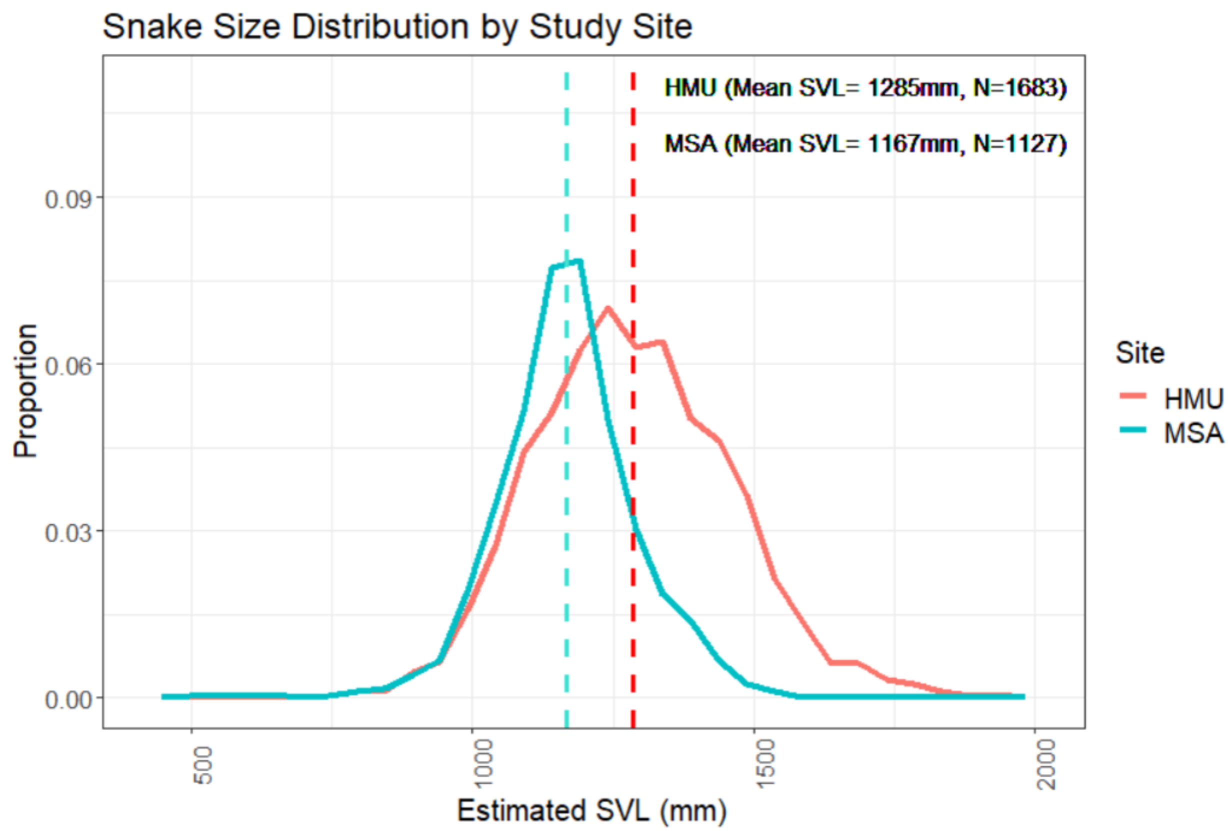


Figure 5a) We plotted the distributions of estimated snake snout-vent-lengths (SVL) in the MSA (blue) and the HMU (red). The observed mean SVL was 1,285mm in the HMU and 1,167mm in the MSA. We estimated snake SVLs from high quality game camera photos using size standards to take snake head size measurements, and allometric regression to estimate SVL from head size measurements. Data was based on 1,683 observations in the HMU and 1,127 observations in the MSA. We evaluated the relationship between snake size and study site using a negative binomial mixed-effects model. and found that the estimated snake size (snout-vent length, or SVL) varied significantly by Study Site ($P<.001$, $\beta(MSA) = -.086$, $SE= .009$) (Table 5b).

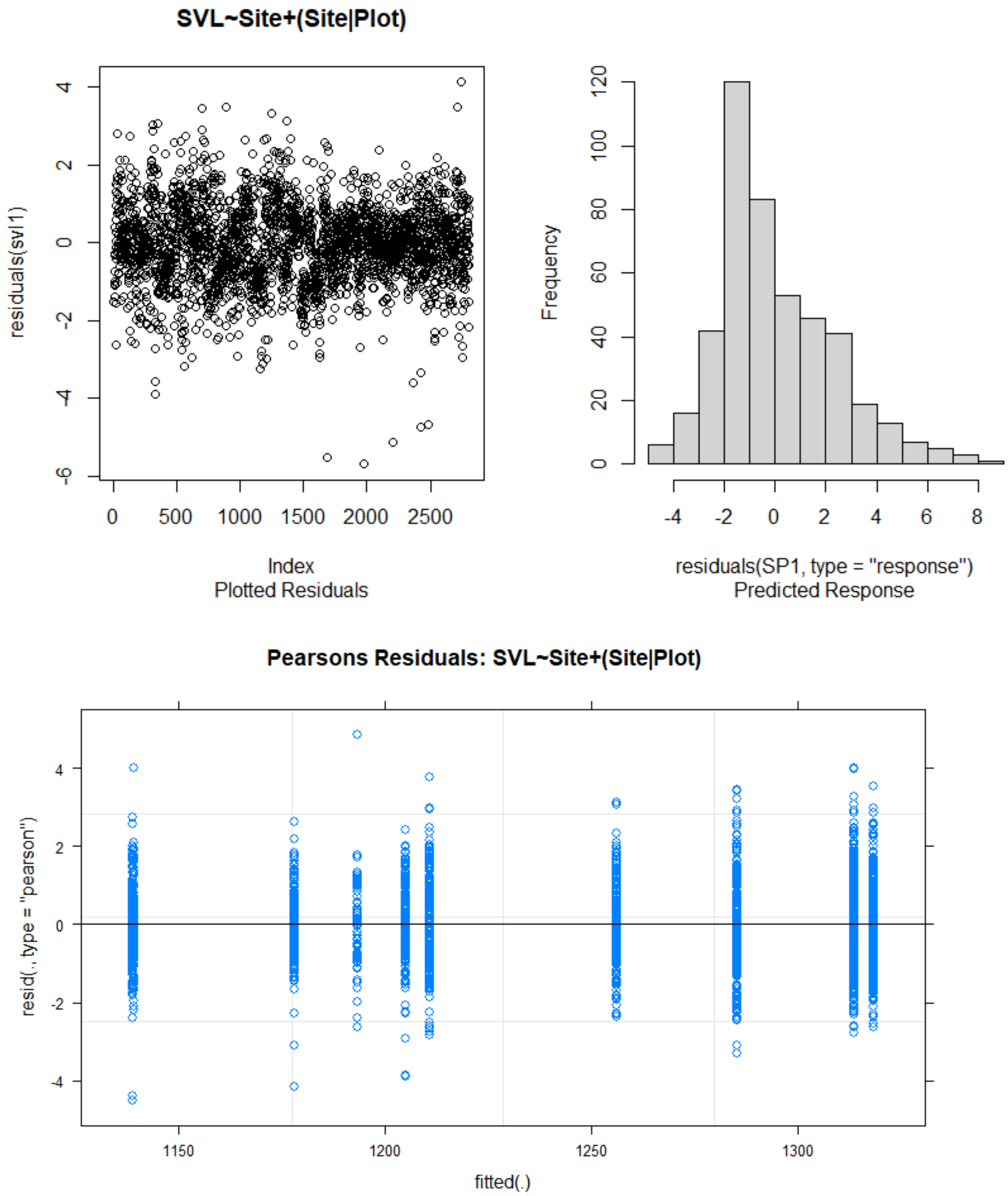


Figure 5b) We modeled expected snake snout-vent-length (SVL) in relation to study site and plotted model residuals. *(Below)* When plotted, Pearson's residuals illustrate that a majority of the residual values lie between y= -2 and y= 2, indicating a typical distribution pattern for a negative binomial regression; (*Top Left*) Points indicate the plotted residuals, which lie mostly between Y=2 and y=-2; (*Top Right)* a Histogram of residual response predictions.

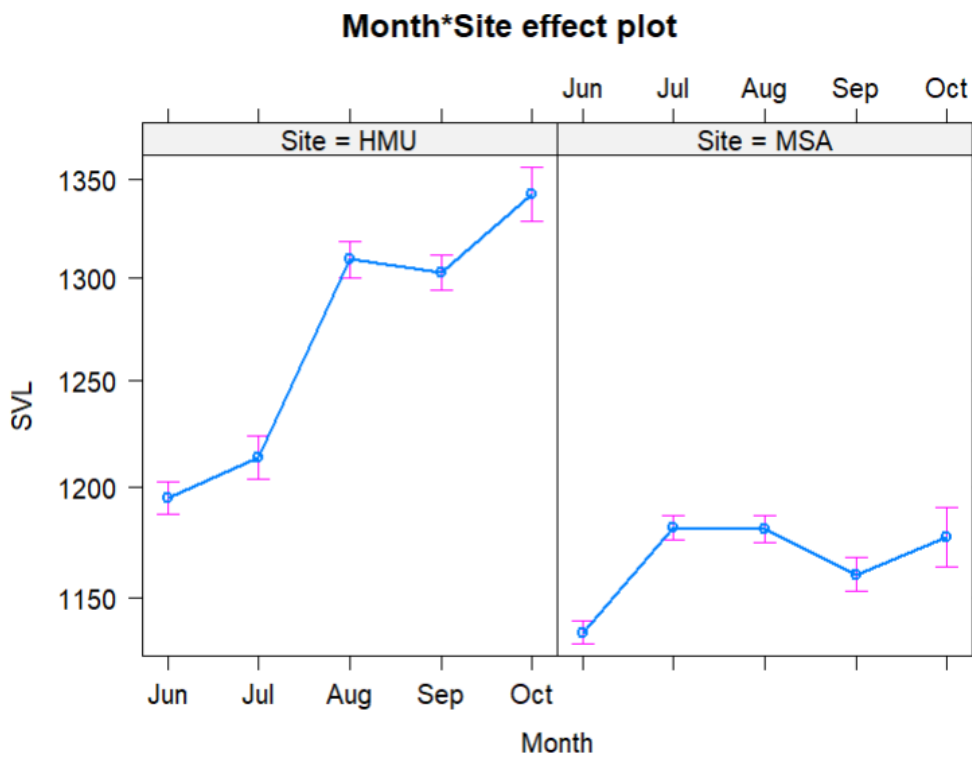


Figure 5c) We modeled expected snake snout-vent-length (SVL) in relation to study site and month following aerial treatment in February 2020. We performed contrasts to compare variance in all factor levels of months and study site (Table 5b). We found that in the treated study site (HMU) expected SVL increased significantly with each passing months since treatment, except for from August to September (P(August)>.05). In the MSA, SVL did not vary significantly from month to month, except for September to October. Points indicate expected snake SVL by month, with lines between points showing the relative slope in SVL change by month, Pink vertical bars on each point indicate error margins (CI=0.95).

Figure 5d)

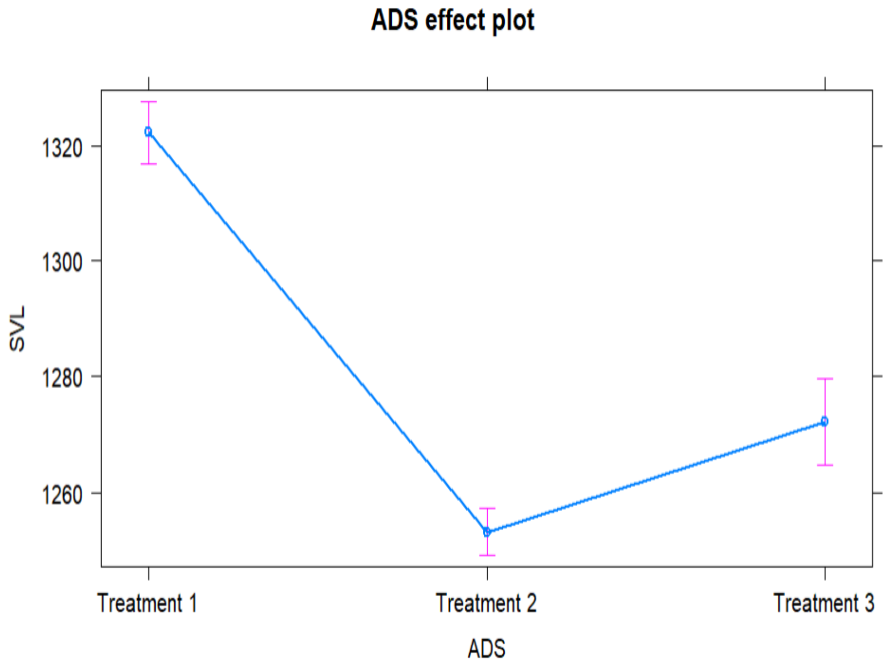


Figure 5c) We used a negative binomial mixed-effects model to evaluate snake snout-vent-length (SVL) in relation to successive treatment applications in the HMU. We used Treatment Status as a fixed effect and grouped data that occurred before, between or after the two treatment applications (here termed ADS Treatment 1, ADS Treatment 2, and ADS Treatment 3). We found that SVL variance was highly significant from Treatment Periods 1 to 2 ($P<.001$, $\beta(2) = -.055$, $SE= .009$), and was significant from Treatment Period 1 to 3 ($P< .05$, $\beta(3) = -.03$, $SE= .014$). The expected SVL was ~1,32ex3mm before, ~1,255 mm between, and ~1,275mm after the two aerial treatments. By comparison, the observed mean SVL was 1,312mm before, 1,243 mm between, and 1,268mm after the two aerial treatments.

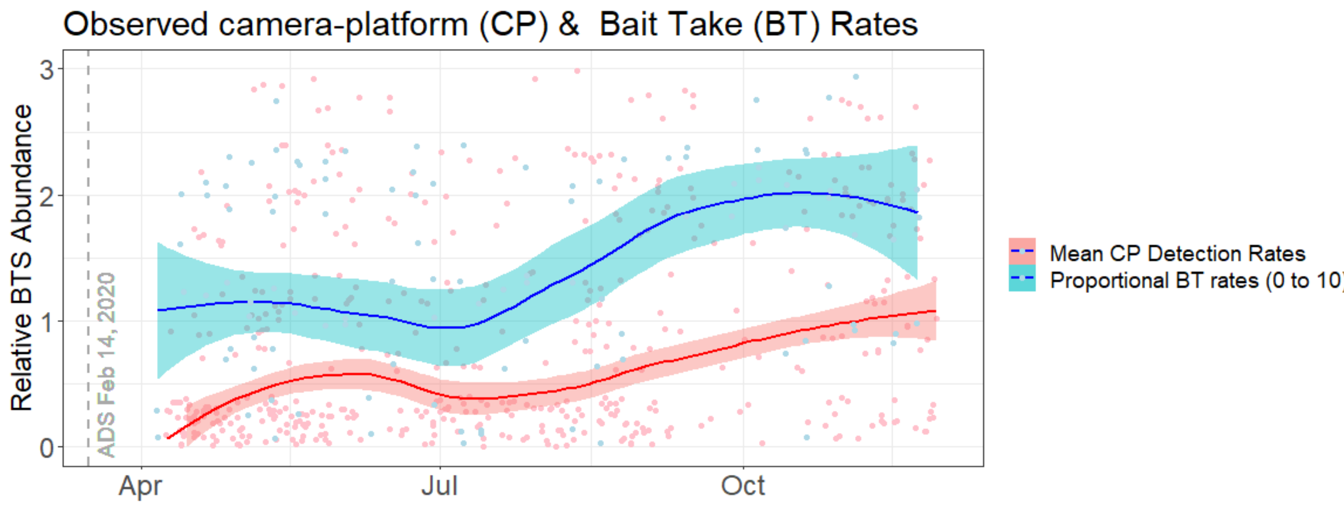


Figure 6a): Observed snake detection rates for bait tube (red) and camera platform (blue) monitoring methods. The points indicate count data for bait takes and for nightly snake detections, respectively. The lines indicate temporal trends in mean detection rates, or relative snake abundance. Smoothing was performed using local weighted means (loess). Shaded areas indicate margin of error (CI=0.95). Mean monthly detection rates for each monitoring method is summarized in Table 6a.

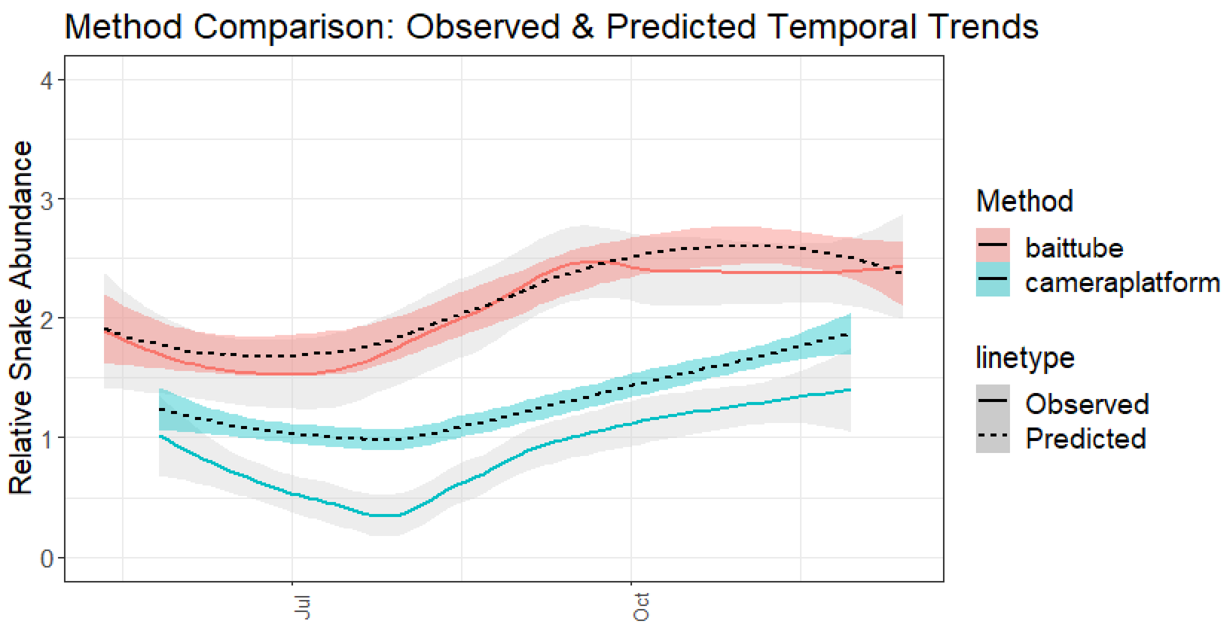


Figure 6b) We plotted model predictions (dashed line) and observed (solid line) snake detection rates for bait tube (red) and camera platform (blue) monitoring methods. We found that both methods demonstrate similar temporal trends in detection rates. The lines indicate temporal trends in mean observed and expected snake detection rates, or relative snake abundance. The dataset used included the pooled count data for bait-tube take rates and for camera-platform snake detection rates. Smoothing was performed using local weighted means (loess). Shaded areas indicate margin of error (CI=0.95). We used a negative-binomial model with fixed effects for time since treatment, and a random effect for trial nested by the method type.

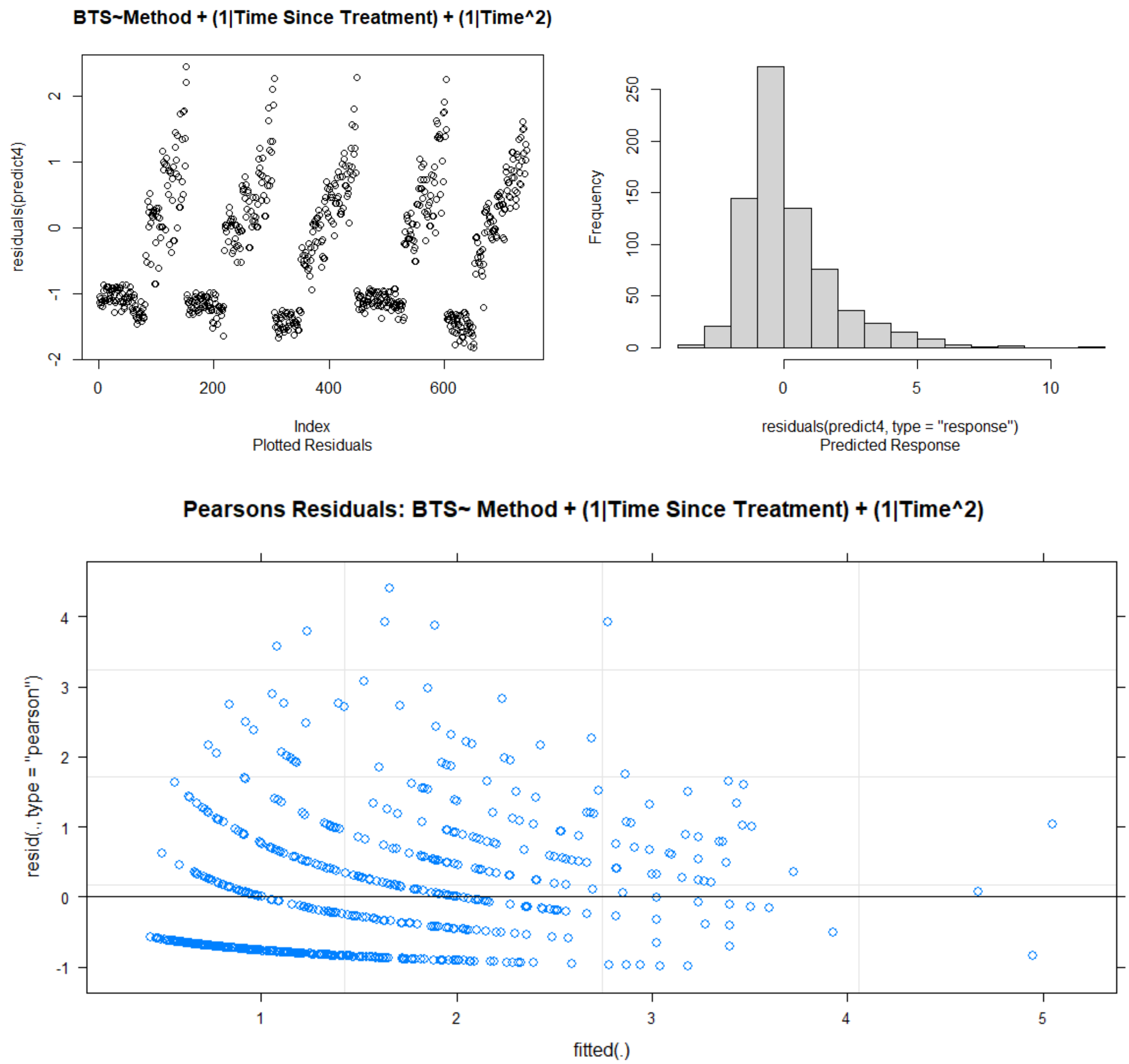


Figure 5c) We plotted snake detection rates for both monitoring methods and included random effects for time since treatment as continuous and quadratic terms. *(Below)* When plotted, Pearson's residuals illustrate that a majority of the residual values lie between y= -2 and y= 2, indicating a typical distribution pattern for a negative binomial regression; (*Top Left*) Points indicate the plotted residuals, which lie mostly between Y=2 and y=-2; (*Top Right)* a Histogram of residual response predictions.

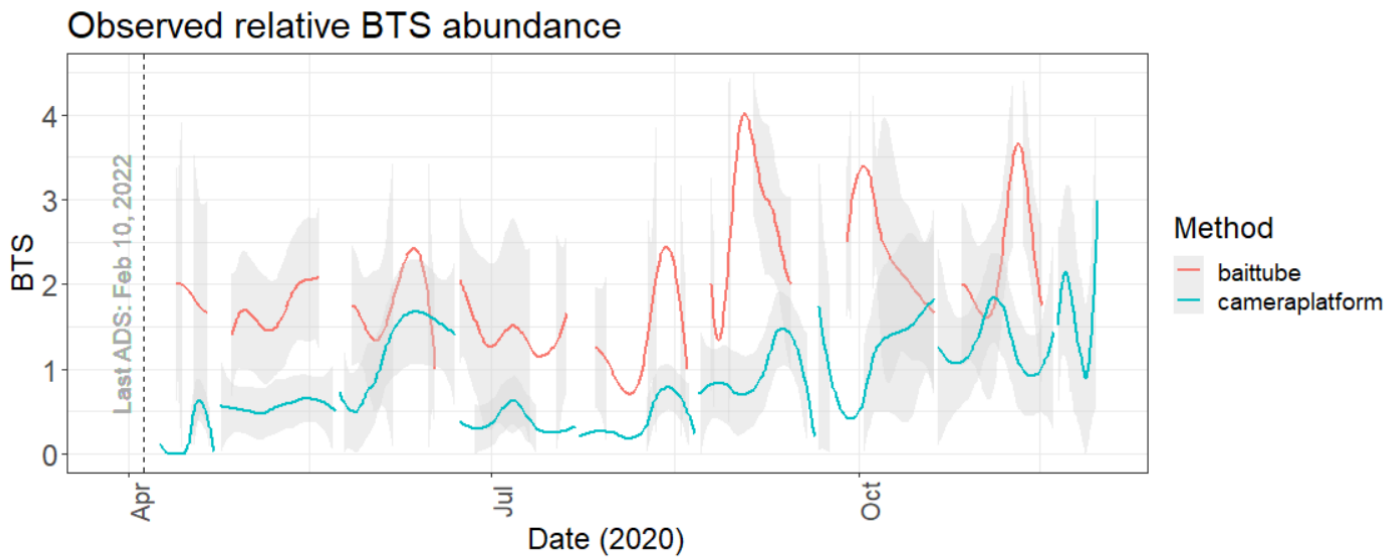


Figure 6d) We plotted the observed snake detection rates for bait tube (red) and camera platform (blue) monitoring methods. The lines indicate temporal trends in mean detection rates, or relative snake abundance. We calculated mean detection rates based on count data for bait-tube take rates and for camera-platform snake detection rates. We applied smoothing to trend detection rates using local weighted means (loess). We evaluated monthly intervals within the monitoring period, applying weighted local means to more refined intervals than before (Figure 6b).

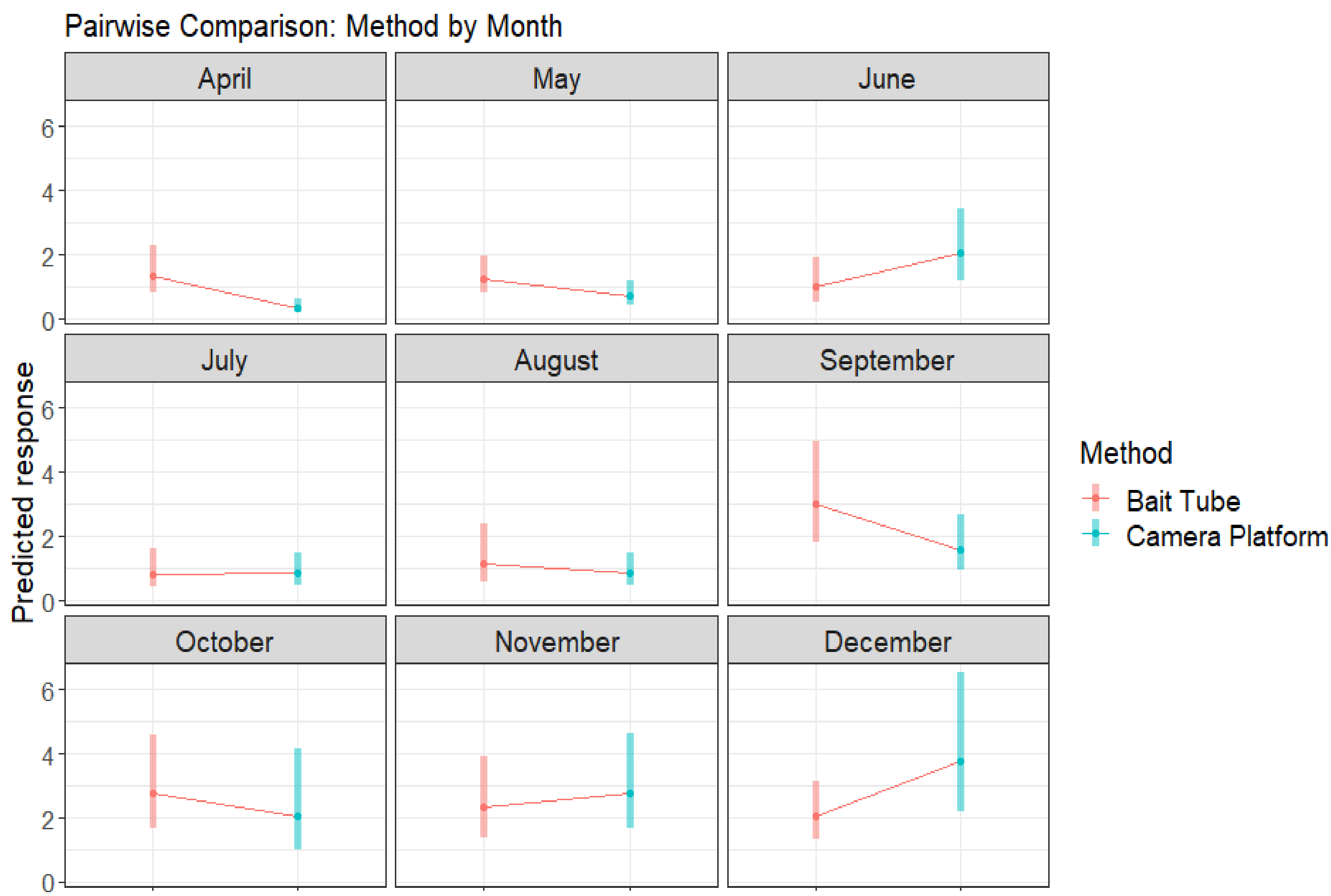


Figure 6e) We modeled snake detection rates in relation to the Month and the Method of data collection. We found that method was not significant (P >.05); Month was significant (P<.001); and that there was significant interaction of method and month (P<.001) (Table 6c). We followed model selection with pairwise comparisons. Contrasts showed that snake detection rates for both methods varied similarly by month; there was no significant variation between camera platform and bait tube detection rates for corresponding months except for in April of 2020 (P > .05 for all months except April) (Table 6d).

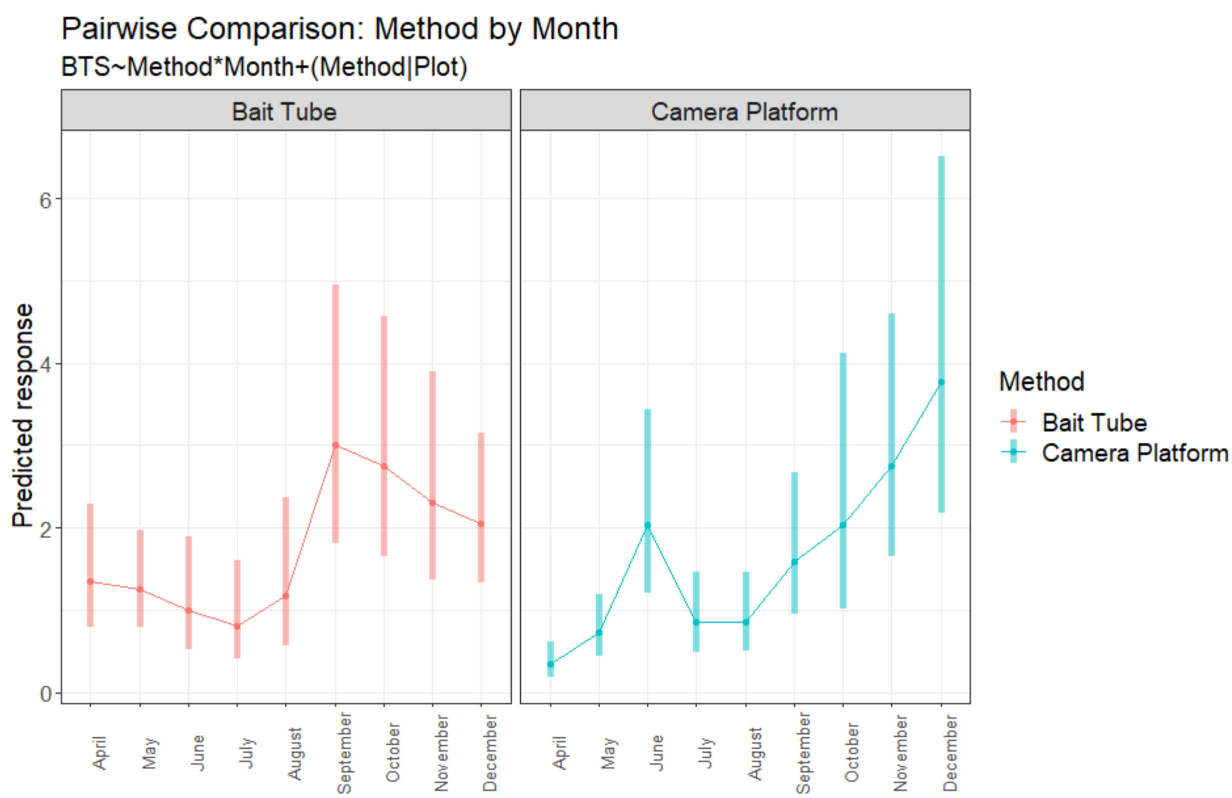


Figure 6f) We applied contrasts to compare month-to-month variation detected by each monitoring method (Table 6e). We found that there were significant increases in detection rates across 7 of the 9 successive months of camera platform data (P< .01 for all months except September and October), but no significant variation across one-month increments in bait tube data (P > .05 for all months). We plotted the pairwise comparisons of snake detection rates by method (Figure 6a). Predictions that were based on camera-platform data illustrated showed a continuous increase in monthly detection rates, with one exception for the month of June 2020. Predictions that were based on bait-tube data showed a pattern of decrease from April to July followed by a spike in detection rates, then a decrease from September to December. These were aligned with the trends detected in the MSA during a study site comparison (Figure 2b).

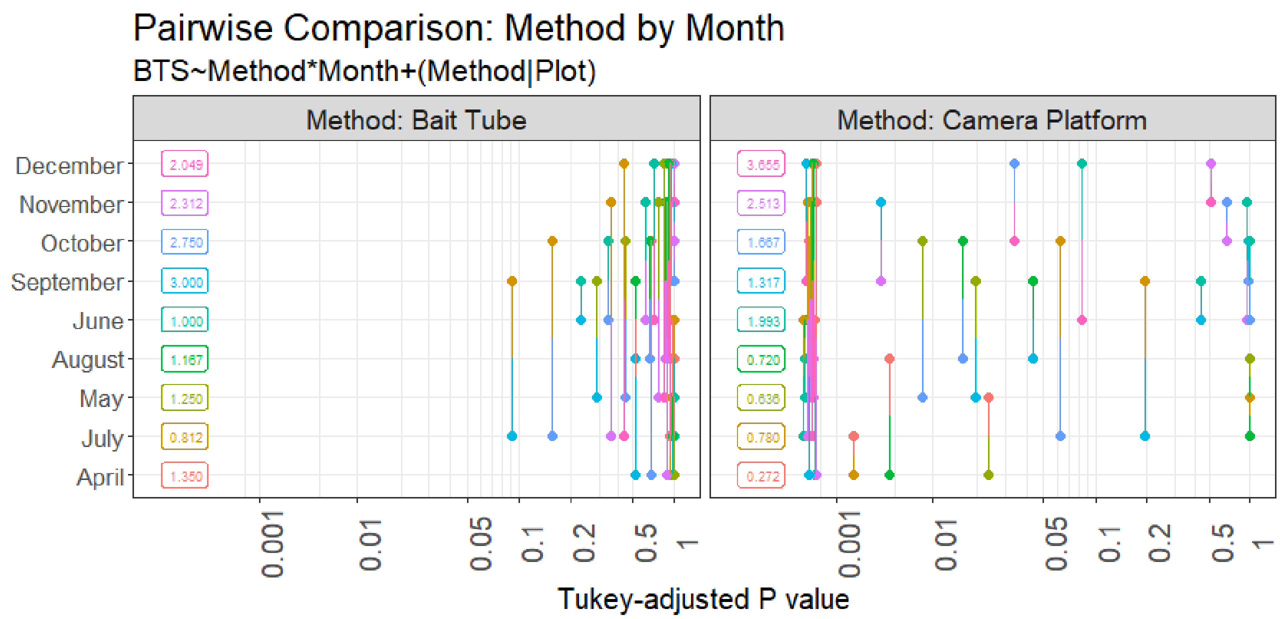


Figure 6g) We plotted the significance levels (Tukey-adjusted p-values) associated with pairwise comparisons of estimated marginal means (Table 6e). We found there were no significant differences in monthly detection rates for bait tube data. For camera platform data, 9 of 36 month-to-month comparisons were statistically similar (P > .05); 5 of 36 were significant (P<.05), and 22 of 36 were highly significant (P<.001). There were significant increases in detection rates across 7 of the 9 successive months of camera platform data (P< .01 for all months except September and October), but no significant variation across one-month increments in bait tube data (P > .05 for all months).

IMAGES

Image 1) Aerial image of Northern Guam shows the study site location, a forested area on a limestone plateau on Anderson Air Force Base. The red box indicates the location of the treated area, the Habitat Management unit (HMU) and adjacent reference site , MSA.

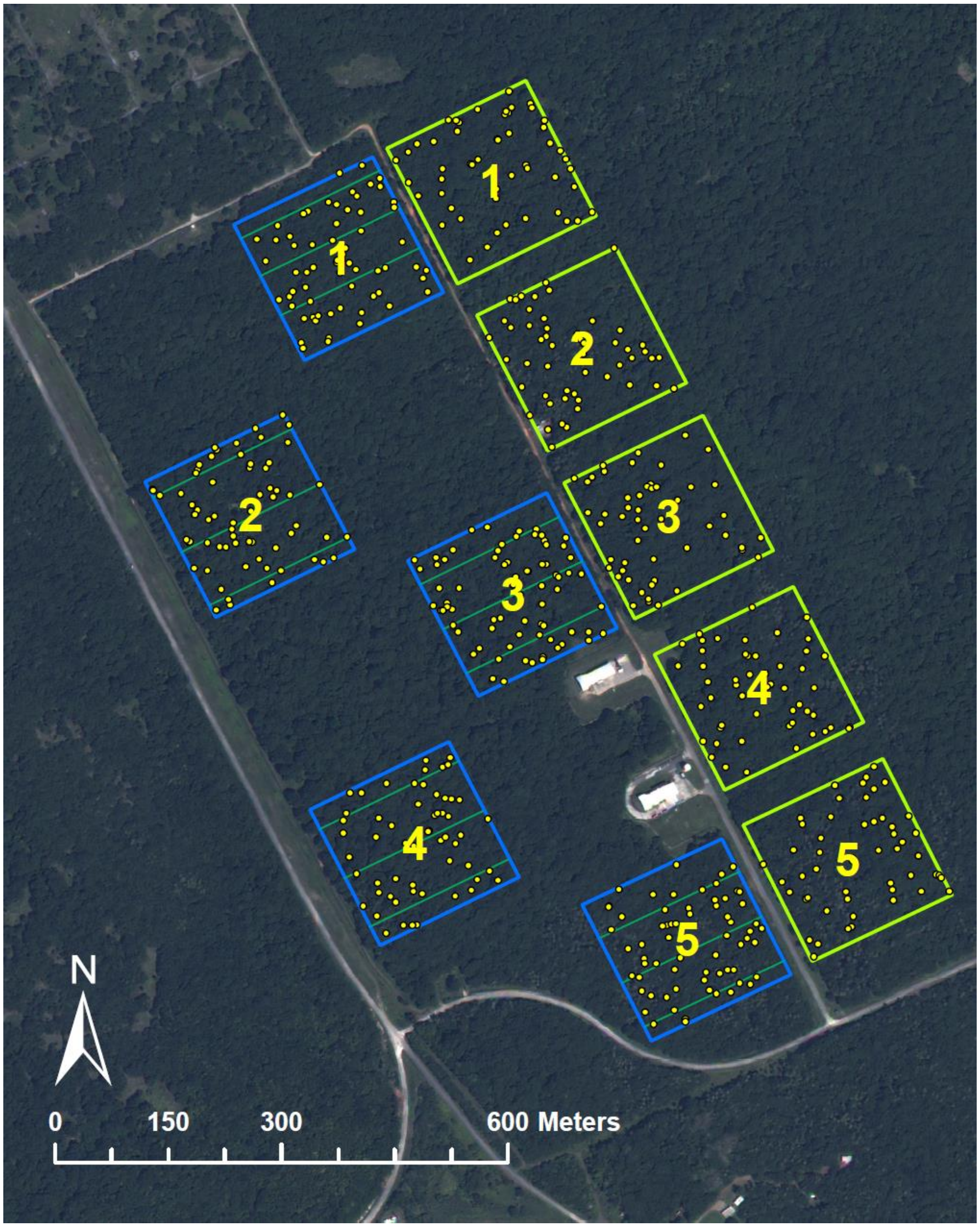


Image 2) Camera platform and bait tube monitoring subplots in the Habitat Management Unit (blue) and the Munitions Storage Area (green), Andersen Air Force Base, Guam. Yellow points represent the list of randomized camera points that were computer generated prior to the study (not all points were actual camera locations).

Image 3) The Aerial Deliver System (ADS) is made up of four magazines carrying 900 bait cartridges each, mounted on an OH-6 helicopter belonging to USDA-WS.

Images 4) The camera platform monitoring method includes an over-head mounted game camera, mounted above a platform printed with size standards for snake size measurements, and

holding a chamber with a live mouse lure (right). The platform can be elevated into the tree canopy using a painter pole, and secured using bungies (right).

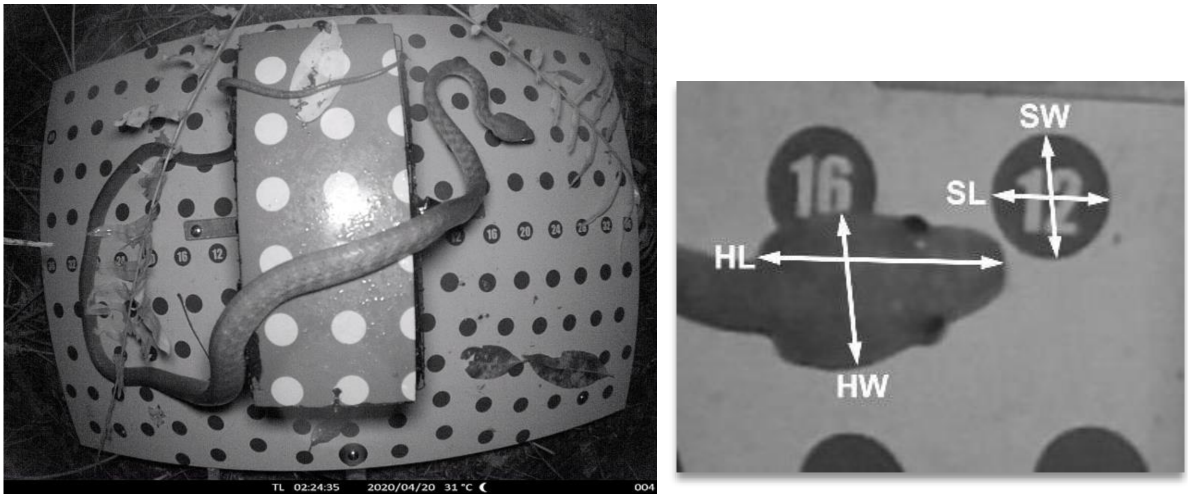


Image 5) Game-camera time lapse photo taken using the camera-platform monitoring method. A brown Treesnake is shown interacting with the live lure, which is placed on a platform that is printed with size standards to aid in taking snake head size measurements. The camera-platform is elevated into the tree canopy and records time lapse photos from 18:00-06:00.

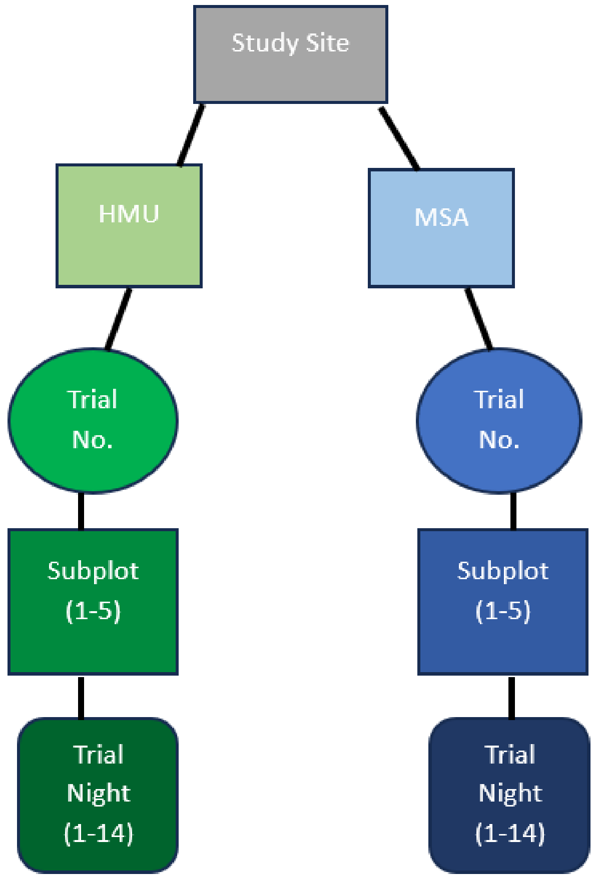


Image 6) Camera-platform monitoring was conducted in two Study Sites (HMU and MSA). Data collection was divided into Trials; Trial Numbers were assigned in sequential order as they occurred but did not always occur simultaneously in both sites. During a Trial, camera-platforms collected data simultaneously in each of five Subplots in a given study site. Camera-platforms were placed at a new location in each Subplot at the start of every Trial, then remained in that location for 14 nights of data collection, or “Trial Nights”.

Appendix B:

Tables, Figures and Images

Evaluation of an Alternative Ground Bait to Supplement Aerial Treatments and Increase Target Species Mortality During Invasive Species Suppression at a Landscape Scale

Figures and tables are included separately from the text, as per submission format guidelines for the Wildlife Society Bulletin: Tools and Technology

FIGURES

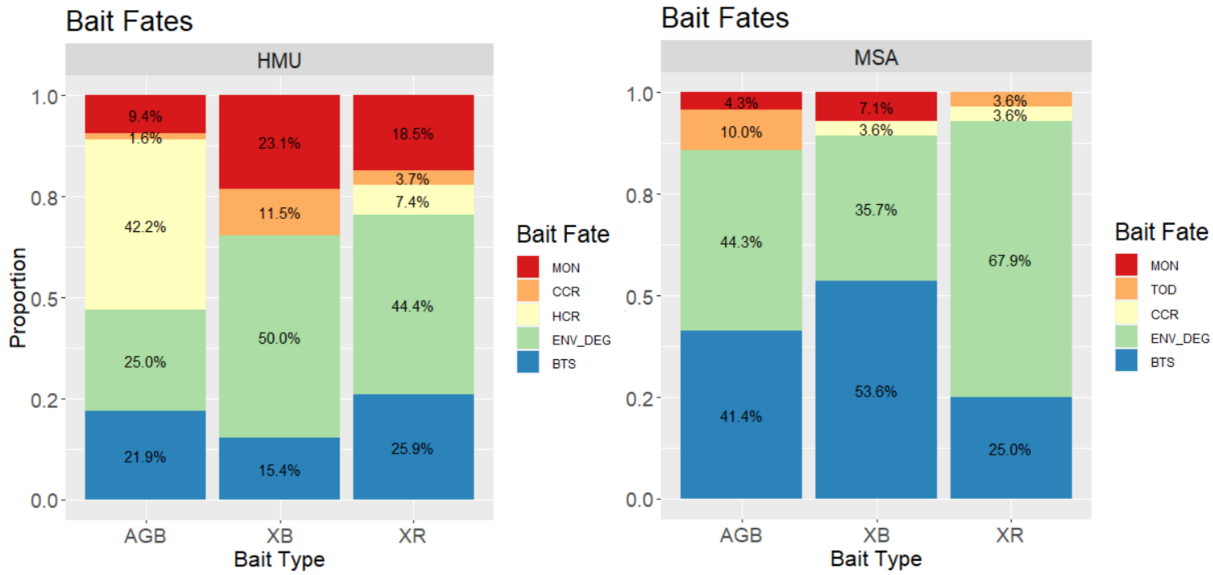


Figure 1) The bar chart demonstrates the proportional outcomes for each of the three bait types tested: alternative ground bait (AGB), large rodent (XR) and large bird (XB). Study sites included the treatment site (HMU; left) and the un-treated reference site (MSA; right). Bait fates are indicated by their color and the code indicated on the legend and are labeled with a percentage which indicates the proportion of a given bait fate for each bait type and study site. Baits fates were determined by game-camera footage by occurrences within a 72-hour monitoring period, which is the maximum time window recommended before bait replacement in current management practice. Species observed included Brown Treesnakes (termed "BTS"), hermit crabs (HCR), Coconut crabs (CCR), toads (TOD) and monitor lizards (MON). Bait fates also include environmental degradation (ENV_DEG), i.e., decomposition or consumption by ants or maggots. Baits of unknown fates were not included in this summary (i.e., the bait was dragged out of the camera frame by a hermit crab, so the fate of the bait could not be confirmed). We also excluded two unique instances (one bait-take by a rat and one by a dog) that are not relevant to our analysis.

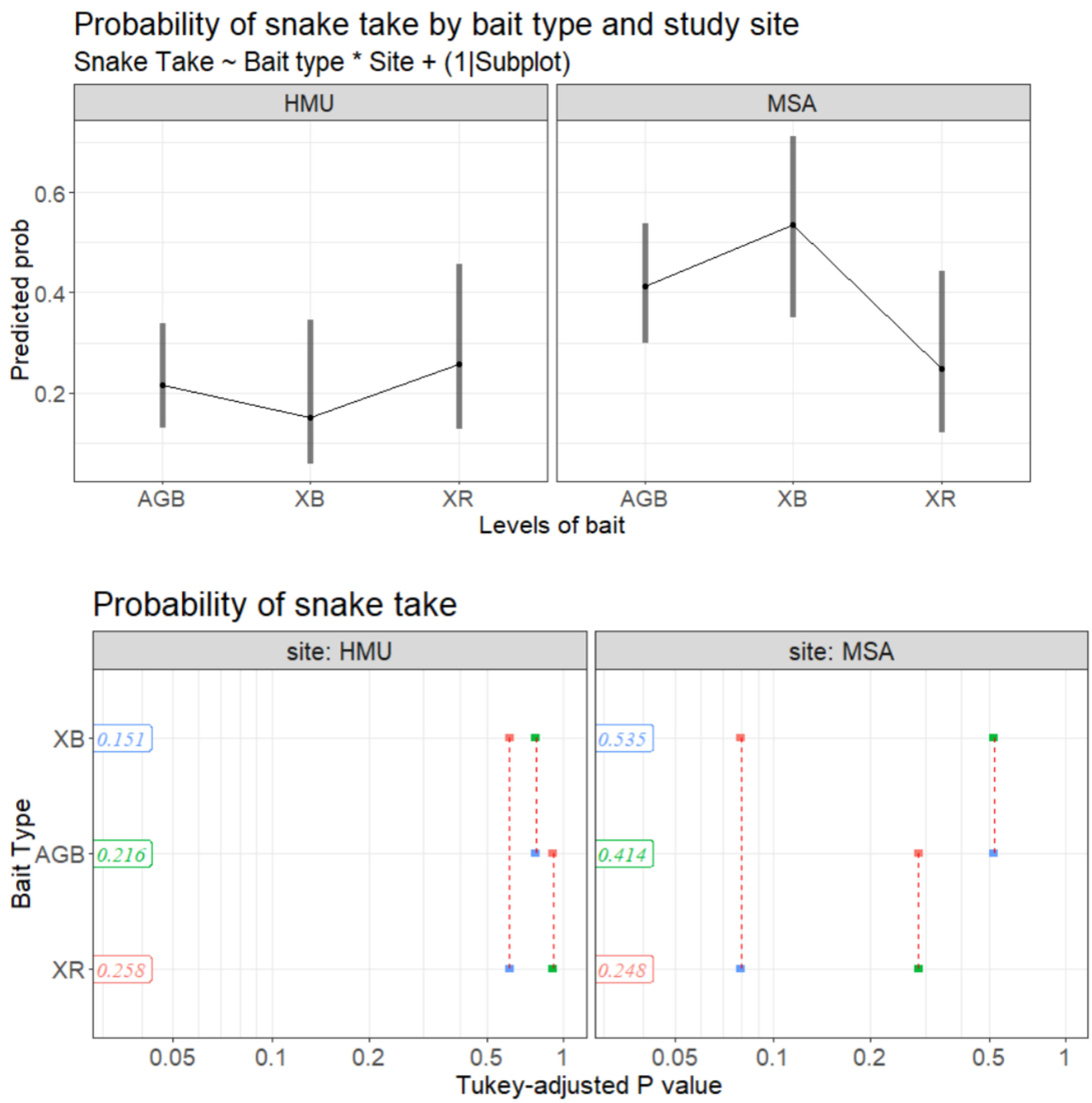


Figure 2) *(Top)* Predicted probabilities of a bait being taken by a snake (y-axis) are plotted for each bait type (x-axis) for both study sites. Bait types included medium rodent (AGB, large rodent (XR), and large bird (XB). Shaded bars indicate margin of error (CI=.95); greater overlap of error bars indicates less significance in outcome by each bait type. Predictions are based on pairwise comparisons of model estimates. *(Below)* Comparisons of bait efficacy among bait types (y-axis) are plotted according to the significance levels (x-axis). We found that within the

HMU there were no significant difference in bait efficacy for any combination of bait types that were compared (P > 0.1 for AGB-XB, AGB-XR, and XB-XR); nor in the MSA P(XB-XR > .05; P(AGB- XR, AGB-XB (P > 0.1). According to model predictions, the probability of a bait being taken by snakes in the HMU was lower overall (XB=15.1%, AGB=21.6%, XR=25.8%) compared to the MSA (XB=53.5%, AGB=41.4%, XR=24.8%). Pairwise comparisons were based on a binomial, mixed-effects linear model.

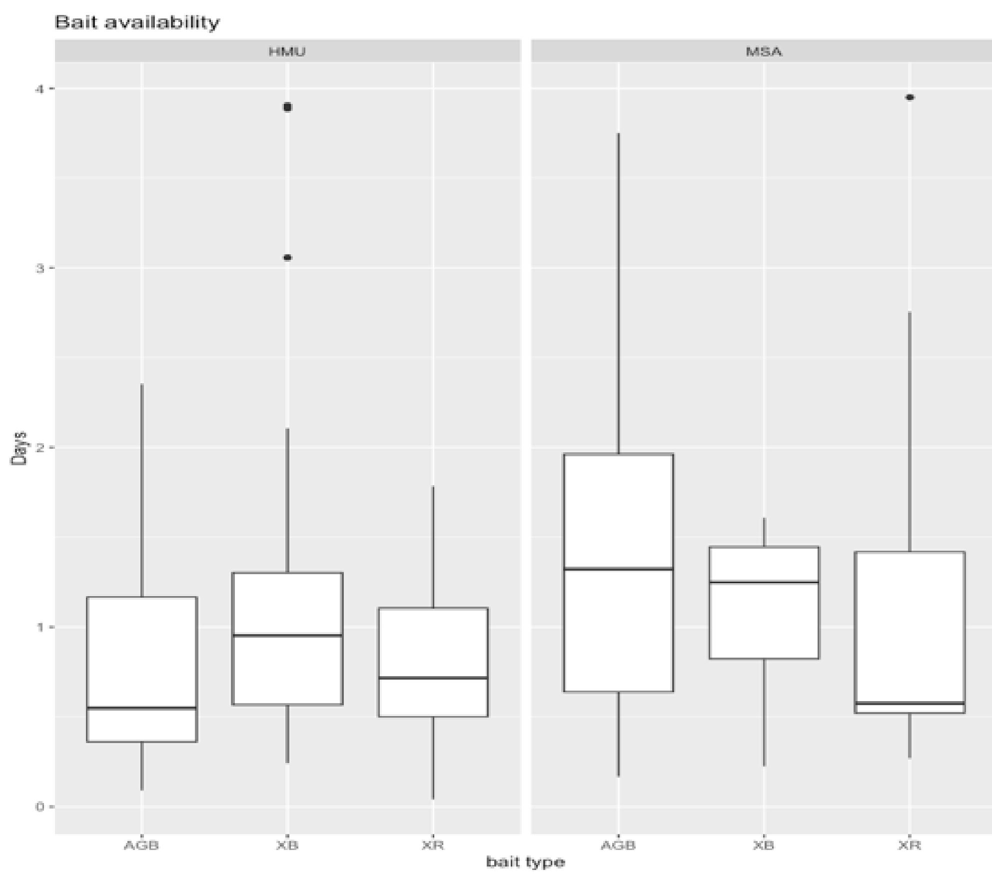


Figure 3) We plotted mean observed bait durations by bait type and study type (Figure 3). On average, AGB baits lasted roughly 0.5 days in the HMU and 1.3 days in the MSA, XB baits lasted roughly 1 day in the HMU and 1.5 days in the MSA, and XR baits lasted roughly 0.75 days in the HMU and .5 days in the MSA.

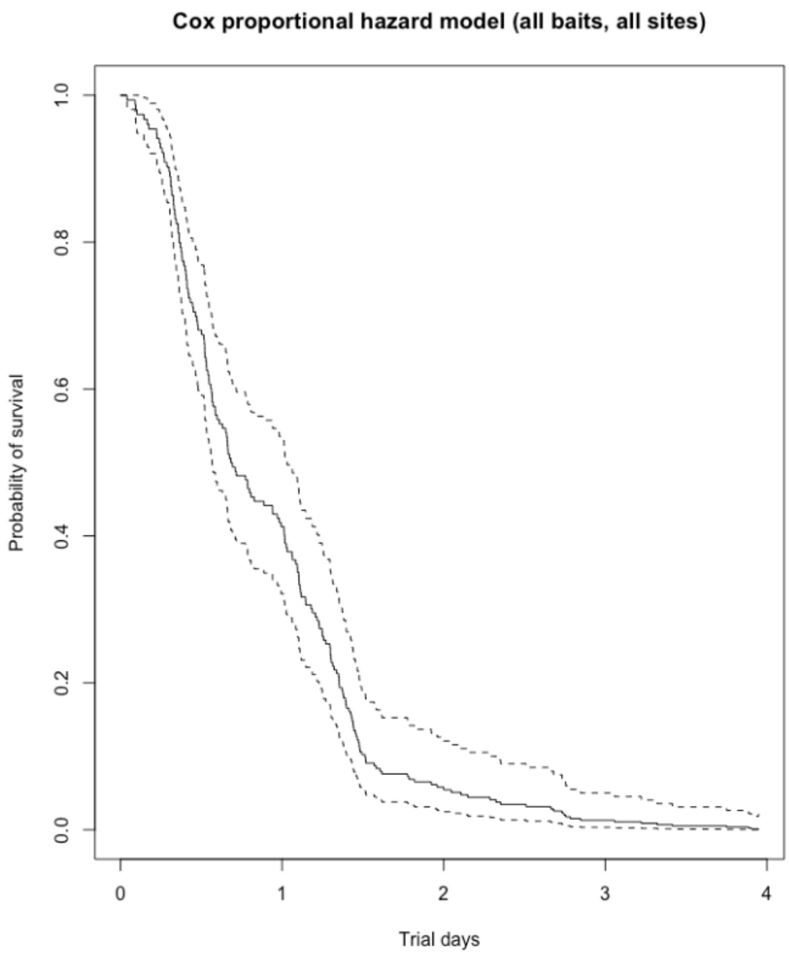


Figure 4) We performed cox proportional hazard analyses and found that across all bait types, the duration of bait availability did not vary significantly by study site (P>0.1). According to the cox proportional hazard model, baits have a 0.4 probability of persisting until 1 day (24 hours) after deployment. This drops to approximately 0.1 probability of survival at approximately 1.5 days (36 hours) after deployment. Few baits remain for 3 days or more

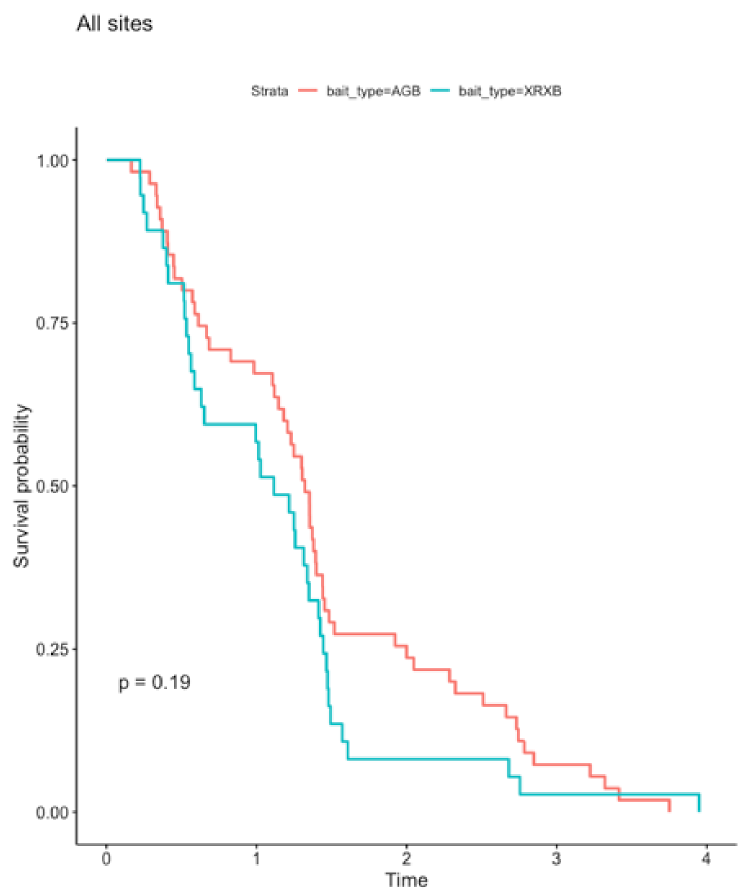


Figure 5) proportional hazard models for survival probability of AGB (red) and non-AGB (blue) baits across all study site data. The predicted survival probability (y-axis) is plotted against time (days, x-axis) of bait persistence in the field. We subjected bait duration to survival analysis using Cox proportional hazard model to predict the probability that a bait (categorized as AGB or Non-AGB) will remain available with increasing time since in the field. We found that survival probability did not vary significantly between AGB and non-AGB baits (P > 0.1).

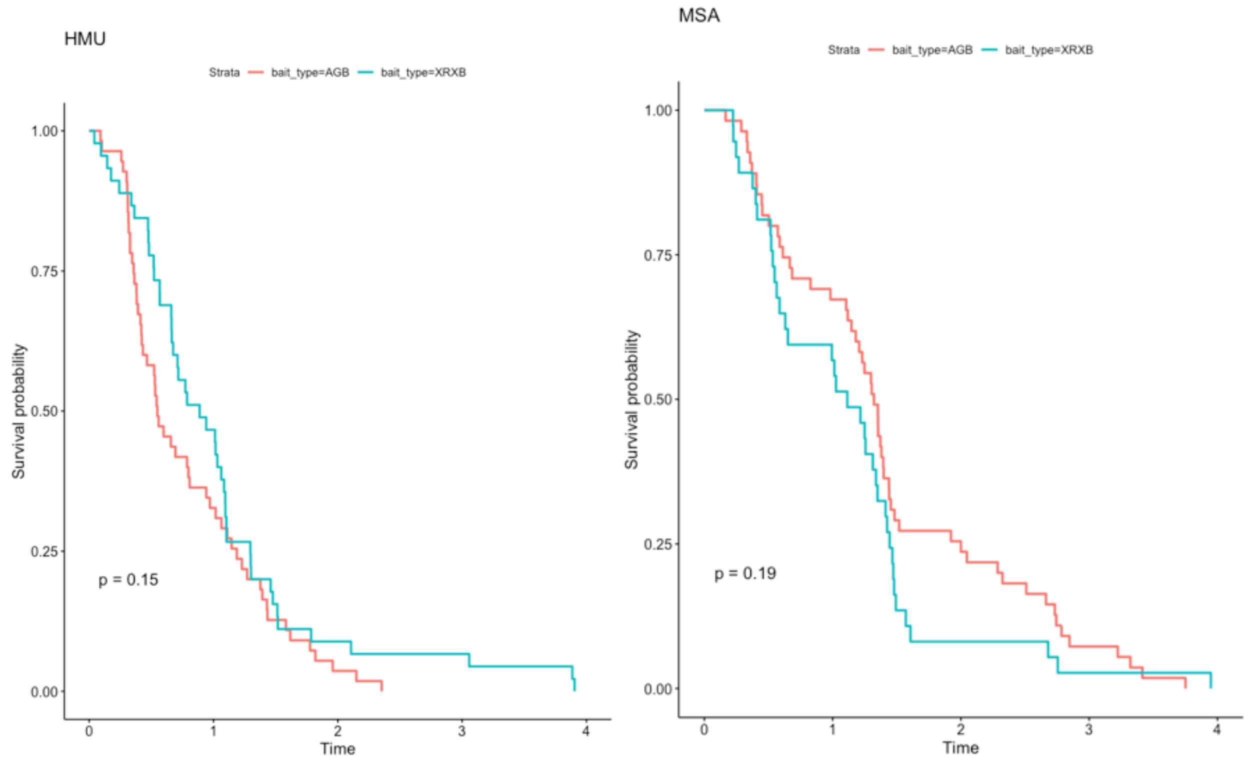


Figure 6) proportional hazard models for survival probability of AGB (red) and non-AGB (blue) baits in HMU and MSA, respectively. The predicted survival probability (y-axis) is plotted against time (days, x-axis) of bait persistence in the field. We subjected bait duration to survival analysis using Cox proportional hazard model to predict the probability that a bait (categorized as AGB or Non-AGB) will remain available with increasing time since in the field. We found that survival probability did not vary significantly between AGB and non-AGB baits in either study site (P > 0.1).

TABLES

Occurrences of "Successful" Bait Fates (Baits taken by BTS)

Bait	#HMU	%HMU	#MSA	%MSA	#Total	%TOTAL
AGB	14	56	29	56.86	43	56.57
XB	4	16	15	29.41	19	25
XR	7	28	7	13.72	14	18.42
XRXB 1	1	44	22	43.13	33	43.42
TOTAL	**25**		**51**		**76**	

Table 1) We summarized frequencies of successful bait fates, i.e. bait fates attributed to Brown Treesnakes (here termed "BTS"). We compared outcomes among across study sites (HMU and the MSA), and among the types of ground baits that were evaluated (AGB= medium mouse bait, XB= bird bait, XR= rat bait, and XBXR= the grouping of non-AGB baits, or rat and bird baits)

Summed Bait Days & Success Rates (# BTS)

Bait	Days (HMU)	Days (MSA)	Total	BTS(HMU)	BTS(MSA)	BTS Totals
XBXR	46.69	41.33	88.02	11	22	33
AGB	44.01	78.66	122.67	14	29	43
TOTAL	90.70	119.99	210.68	25	51	76

Table 2) We calculated "bait days" as the duration of time a bait remained in the field across. We totaled the associated frequency of successes, or bait fait by Brown Treesnake here termed "BTS"). We compared outcomes among across bait (AGB, or medium mouse baits, and the grouping of non-AGB baits "XBXR, or rat and bird baits) types and study sites (HMU and the MSA).

CPUE (# BTS/100Baitdays)

Bait	HMU	MSA	TOTAL
XRXB	24	53	37.5
AGB	32	37	35
TOTAL	28	42.5	36

Table 3) We calculated "CPUE" as the frequency of successes (bait fait by Brown Treesnake) divided by the summed bait days. We compared outcomes among across bait (AGB, or medium mouse baits, and the grouping of non-AGB baits "XBXR, or rat and bird baits) types and study sites (HMU and the MSA).

IMAGES

Image 1) Aerial image of Northern Guam shows the study site location, a forested area on a limestone plateau on Anderson Air Force Base. The red box indicates the location of the treated area, the Habitat Management unit (HMU) and adjacent reference site , MSA.

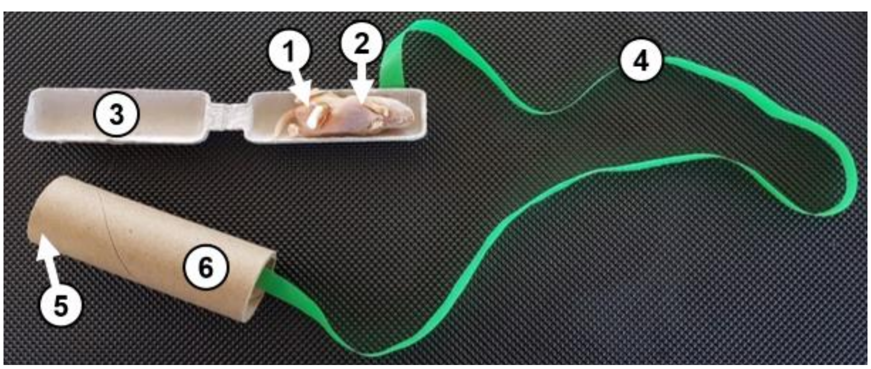


Image 2) A typical ADS bait is a "fuzzy" mouse (4-6g). Components of an aerial delivery system (ADS) bait cartridge: 80-mg acetaminophen tablet (1) adhered to a dead newborn mouse bait (2)

that is partially glued into the bait capsule (3); the capsule is folded around the bait and wound with a ribbon (4) which is attached to an end cap (5, not seen); the outer tube (6) is fitted over the wound capsule and end cap, for a completed bait cartridge.

Image 3) Internal components of the bait cartridge (Figure 1) may be replaced with a larger 13-17-g mouse with tail and hind limbs removed, with no capsule or ribbon for canopy entanglement. Instead, two endcaps would keep the mouse encased while in the automated dispensing module (ADM) magazine.

Image 4) Ground baits included chicks (pictured) and large rodents. All baits were deployed on the forest floor, where their fate was monitored by an overhead-mounted game camera.

Image 5) Overhead-mounted game cameras were used to monitor fate of ground-deployed baits. The tripod was fashioned from electrical conduit pipe.

MEMORANDUM

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SUBJECT: THESIS

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Student's Name: Ella Leona Rika Norris

Title of Thesis: Changes in relative abundance and size distributions of invasive Brown Treesnakes during landscape-scale aerial baiting using novel and standard monitoring methods, and alternative baits for targeting large snakes

Number of Pages: 167

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