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Shane R. Siers

Scott M. Goetz

Rachel M. Volsteadt

Melia G. Nafus

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Research Article

Evaluating lethal toxicant doses for the largest individuals of an invasive vertebrate predator with indeterminate growth

Shane R. Siers[1,*], Scott M. Goetz[1,†], Rachel M. Volsteadt[1] and Melia G. Nafus[2]

[1]*U.S. Department of Agriculture, Wildlife Services, National Wildlife Research Center, 233 Pangelinan Way, Barrigada, GU, USA*
[2]*U.S. Geological Survey, Fort Collins Science Center, Fort Collins, Colorado, USA*
[†]*Current affiliation: U.S. Geological Survey, Fort Collins Science Center, Fort Collins, Colorado, USA*
Author e-mails: *shane.r.siers@usda.gov (SS), sgoetz@usgs.gov (SG), rachel.m.volsteadt@usda.gov (RV), mnafus@usgs.gov (MN)*
**Corresponding author*

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Abstract

The brown treesnake (*Boiga irregularis*) was accidentally introduced to Guam and caused severe ecological and economic damages. Acetaminophen is an effective, low-risk oral toxicant for invasive brown treesnakes, and an automated aerial delivery system (ADS) has been developed for landscape-scale toxic bait distribution. A fixed dose of 80 mg of acetaminophen within a tablet inserted into a dead neonatal mouse (DNM) was lethal for all brown treesnakes in previous trials; however, these trials did not include very large individuals which are difficult to acquire for testing. Because most reptiles continue to grow throughout their lifespan, a small number reach much greater than average body sizes. Here, we tested effectiveness of 80 mg acetaminophen DNM baits for unusually large brown treesnakes as they became available. Our results confirmed that an 80 mg dose is lethal for the vast majority of snakes on Guam, but efficacy starts to diminish around 200 g of body mass. We also tested an alternative mouse bait configuration with 160 mg of acetaminophen that could be incorporated into the ADS to improve control of unusually large snakes. The 160 mg dose is expected to be effective for nearly all female snakes; males grow much larger and additional methods will be needed for extraordinarily large individuals. We describe a full dose-response curve for brown treesnakes to acetaminophen tablets and estimate the LD_{90} at 299 mg/kg and the LD_{99} at 578 mg/kg. To our knowledge, this is the first published dose-response curve for an invasive vertebrate with indeterminate growth.

Key words: acetaminophen, aerial baiting, brown treesnake *Boiga irregularis,* eradication, invasive alien species, vertebrate pesticide

Introduction

Oral toxicants are an important tool for vertebrate pest control and invasive vertebrate management. The dose/response relationship of oral toxicity generally dictates that individuals with greater mass require more of a substance to induce lethal intoxication. For most oral toxicants, the amount of active ingredient administered to a target pest animal is modulated by its concentration in a toxic bait and how much the target animal will voluntarily consume. For some oral toxicants, the amount of active ingredient administered is necessarily fixed because it is consumed in a single bolus dose.

Mammals and birds have been the primary target organisms for development and use of oral vertebrate toxicants. Mammals and birds exhibit determinate growth, wherein they stop growing when they reach a predictable and limited size range shortly after sexual maturity. Mammals and birds are endotherms that require frequent feeding to fuel their metabolic demands. Most mammal and bird pest species are herbivores or omnivores for which development of a plant-based bait matrix is usually relatively straightforward.

Other vertebrate pests, such as reptiles and fishes, exhibit indeterminate growth in which they continue to grow throughout their ontogeny (Hariharan et al. 2016). When testing oral toxicant efficacy for species with indeterminate growth, testing should take into account that the amount of toxicant necessary to kill all adults will vary greatly. Reptiles are ectotherms; their lower metabolic rate reduces caloric requirements and enables them to forego feeding for prolonged periods (Pough 1983). At the extreme, snakes are adapted to taking very large meals at infrequent intervals (Mushinsky 1987; Arnold 1993; Siers et al. 2018). Because many invasive reptiles are obligate carnivores, developing a stable bait matrix can be more technically challenging.

As long as the toxic bait can be delivered in a species-appropriate manner, mammal and bird vertebrate pests can often be effectively controlled with an easily-formulated bait on which they will reliably and repeatedly feed, and larger animals will typically consume more bait ensuring that even the largest individuals within a relatively narrow size range will consume a lethal dose. Because reptiles, especially snakes, can forego feeding for longer periods, control of many invasive reptiles will typically require that a lethal dose be delivered within a single feeding on an animal-based bait item. It is desirable that a single dose be sufficient to kill even the largest of individuals within a very broad size range, particularly if the objective is eradication.

The brown treesnake (*Boiga irregularis* Merrem, 1802) is a mildly-venomous, nocturnal, arboreal, generalist predator that was accidentally transported from the Admiralty Islands to the formerly snake-free island of Guam in shipments of military equipment near the end of World War II; by the late 1980s the brown treesnake had invaded all terrestrial habitats on the island and achieved densities unprecedented for any non-aggregating snake species (Rodda and Savidge 2007). Simultaneous with the spread of brown treesnakes across Guam was a wave of ecological and socioeconomic damages including: extinction or extirpation of nearly all native forest birds, with cascading ecological consequences; bites to humans and predation on domestic animals; costly damage to the island's power infrastructure; and millions of dollars in annual expense for measures to prevent spread of brown treesnakes from Guam to other islands (Savidge 1987; Rodda and Savidge 2007; Caves et al. 2013; Clark et al. 2018).

Figure 1. Brown treesnakes can vary tremendously in size. Pictured are a small juvenile (713 mm snout-vent length, 36 g body mass; left) and an unusually large male (1,800 mm, 1,285 g; right). Photo: S. Siers.

Like most arboreal snakes, the brown treesnake is relatively small and slender. On Guam, the majority of individuals are less than 1,000 mm snout-vent length (SVL) and 100 g in body mass. Females on Guam rarely exceed 1,200 mm and 250 g, but extraordinarily large males can grow to over 2,000 mm SVL and 2,000 g (Savidge 1991; Siers et al. 2017; Figure 1). A brown treesnake's size influences nearly every aspect of its biology, ecology, behavior, and susceptibility to invasive species management tools, as well as their potential to spread and cause further damage (Siers et al. 2017; Clark et al. 2018).

The U.S. Department of Agriculture (USDA) Wildlife Services National Wildlife Research Center (WS-NWRC) has screened multiple chemicals for efficacy as brown treesnake dermal and oral toxicants and discovered that uncoated tablets ranging from 40 to 325 mg of acetaminophen, inserted into dead neonatal mice (DNM) as a bait matrix, could achieve 100% mortality when ingested by brown treesnakes (Savarie et al. 2000; Savarie 2002). Also known as paracetamol, acetaminophen is a common human analgesic available over the counter in most countries. Snakes are extremely sensitive to acetaminophen toxicity because they lack gene coding for detoxification enzymes, resulting in the impairment of liver function, muscle damage, and accumulation of acetaminophen metabolites that cause methemoglobinemia and subsequent expiration of snakes due to severe hypoxia, with little sign of pain or distress (van den Hurk and Kerkkamp 2019; Mathies and Mauldin 2020). Following a risk assessment (Johnston et al. 2002), an uncoated tablet product containing 80 mg of acetaminophen (72.73% w/w) was registered with the U.S. Environmental Protection Agency (USEPA) as a toxicant for brown treesnakes under the product name "Acetaminophen for Brown Treesnake Control" (Registration

Figure 2. Left: standard aerial delivery system (ADS) bait with 4 to 6 g dead mouse bait and one 80 mg acetaminophen tablet adhered externally. Right: proposed alternative ground bait comprising a 13 to 17 g dead mouse with two 80 mg tablets inserted into the body. Photo: S. Siers.

No. 56228-34) in 2003. Acetaminophen has subsequently been tested as a potential oral toxicant for other invasive reptiles (reviewed in Friebohle et al. 2020; Kraus et al. 2020).

With funding from the U. S. Departments of the Interior and Defense, WS-NWRC partnered with a private engineering firm (Applied Design Corporation, Boulder, Colorado) to develop an automated system for the assembly and aerial delivery of acetaminophen baits for landscape-scale brown treesnake suppression (Siers et al. 2019, 2020a, b). The core of the aerial delivery system (ADS) is a patented biodegradable bait cartridge (Messaros et al. 2017) comprising a 4 to 6 g DNM with the registered 80 mg acetaminophen tablet adhered to the abdomen (Figure 2). Upon ejection from the helicopter-mounted dispensing module, the cartridge opens in mid-air unfurling a ribbon for entanglement in the canopy and exposing the bait for consumption by brown treesnakes.

The ADS is currently being employed in an experimental brown treesnake eradication attempt within a 55 ha snakeproof barrier and has been shown to achieve drastic reduction of snake abundance (Siers et al. 2020a, b). While apparently extremely effective at removing mid-sized brown treesnakes, several lines of evidence have demonstrated that the current baits may be less effective at removing larger snakes (Nafus et al. 2020a, b; Goetz et al. 2021). Hypotheses for reduced effectiveness in larger brown treesnakes include reduced attractiveness of carrion to larger snakes (Shivik and Clark 1999), greater frequency of terrestrial foraging by larger snakes (Rodda and Reed 2007; Siers 2015; Nafus et al. 2020), and inadequacy of an 80 mg dose of acetaminophen within a DNM to reliably dispatch very large snakes (Nafus 2021).

In past trials, tablets containing 80 mg of acetaminophen in a DNM bait proved lethal to all test animals when ingested (N = 47); however, the largest female tested weighed only 240 g, and the largest male was 320 g (Savarie 2002). Larger snakes are very rare and difficult to obtain for testing. Snakes greater than 320 g comprise only 3.4% of snakes on Guam (see "reference population" in Methods), and a toxicant dose that is reliably lethal for 96.6% of an invasive vertebrate population is extraordinarily effective. However, larger brown treesnakes are a significant demographic with respect to potential damages and challenges for eradication. Larger snakes prey on a wider range of prey sizes, including native and domestic animals, and can inflict more serious bites to humans (reviewed in Siers et al. 2017). Larger females tend to have more enlarged ovarian follicles (Nafus et al. 2020b), which is likely to lead to larger clutch sizes as is documented for other snakes (Madsen and Shine 1996; Shine et al. 1998). The impacts of large brown treesnakes are disproportionate to their low frequency within in the overall population.

In Phase 1 of this study we evaluate the limitations of the standard ADS bait (DNM with EPA-approved 80 mg acetaminophen tablet) for lethal control of the largest brown treesnakes. When it became clear that some very large snakes could survive multiple 80 mg acetaminophen doses, we implemented Phase 2 in which we evaluated a slightly larger bait treated with two 80 mg acetaminophen tablets that could also be dispensed by the ADS. Lastly, we pooled all previous brown treesnake acetaminophen dosing data to more fully describe the dose-response curve and other aspects of acetaminophen performance as an oral toxicant for control of this harmful invasive predator. Throughout this article, the current and previous studies are referred to by the WS-NWRC protocol quality assurance (QA) tracking number (e.g., the current study is referred to as QA-2927).

Materials and methods

Specimen collection and reference population

Dosing trials are typically conducted in a single effort, with 10 to 20 individuals treated simultaneously. However, it has not been possible to gather a large sample of very large brown treesnakes to test in this manner. Instead, we maintained an outreach effort to cooperating agencies and the public, informing them of research interest in very large snakes (sources listed in Table 1). Some snakes contributed by USGS researchers had undergone previous behavioral trials. As snakes were obtained, we initiated the trial sequence so that individual trials overlapped and were staggered in time.

Throughout this paper we refer to snake sizes as they compare to the distributions of snake lengths and weights for the Guam population of brown treesnakes in general ("reference population"). These distributions are based on the results of a previous systematic, stratified sampling of snakes

Table 1. Sources of large snakes included in this study and the number of snakes per study phase.

Source	Phase 1	Phase 2
USDA Wildlife Services operational program	47	22
USGS Brown Treesnake Lab	5	2
Public donation	3	6
University of Guam Yigo Field Station	1	1
No collection information	9	6
Total	56	31

from 3 replicates each of 6 land cover types: 4 forest types, savanna complex, and urban residential areas. At each site, snakes were hand-captured during nighttime visual encounter surveys; although not completely unbiased, this is the only sampling method for brown treesnakes that puts all size classes at risk of capture (Christy et al. 2010). From 2010 to 2012, each of the 18 sites was searched until a target of 100 snakes per site were captured (N = 1,814 total). Survey details and results are more completely described in Siers 2015 and Siers et al. 2017.

Acclimation and housing

Upon receipt, we recorded: SVL (mm) by stretching the snake along a flexible tape; body mass (g) with either a spring scale or electronic balance; and sex as determined by probing for inverted hemipenes (per Reed and Tucker 2012). We housed snakes in 90 × 60 × 45 cm galvanized wire mesh cages with 1.25 cm mesh spacing (Bass Equipment Company, Monett, Missouri) outfitted with a dowel or tree branch for "arboreal" perching, a length of plastic tube for hiding, and a large pet dish filled with water for drinking and partial soaking. Cages were kept outdoors in Guam's ambient environmental conditions and positioned under a roof, with shade cloth walls preventing direct sunlight exposure. Guam's monthly mean temperatures range from 26.7 °C in January to 27.8 °C in June, and temperatures rarely exceed 32.2 °C during the daytime or fall below 21.1 °C at night; relative humidity ranges from 65–80% in the daytime and 85–100% at night (WERI 2020). Ambient conditions were representative of the environmental conditions and natural diel cycle experienced by free ranging brown treesnakes on Guam.

We acclimated snakes for at least one week prior to dosing. On the first Monday or Tuesday of captivity, we offered each snake one unadulterated dead mouse to ensure that the snake was eating. If the snake refused to eat for 48 hours, another mouse was offered on the following week. If a snake refused to eat for three weeks, it was euthanized and removed from the study (two non-feeding snakes were removed under this practice). A snake was therefore held for 1–3 weeks prior to being offered a dosed bait.

Dosing trials

During Phase 1, we tested female snakes of 150 g body mass or greater and males 250 g or greater. At the beginning of the week following acclimation, we offered each snake a 4 to 6 g DNM with a single external 80 mg

acetaminophen tablet (the registered formulation) adhered to the abdomen with hot melt adhesive, simulating the automated manufacturing of DNM baits currently contained in ADS bait cartridges (hereafter "80 mg ADS bait"; Figure 2, left). If the bait was not consumed by the snake within 48 hours, we removed it and offered another bait at the beginning of the following week. If the snake refused the bait for three consecutive feeding attempts, it was euthanized.

After the bait was consumed, we checked snakes at least three times per day, approximately every 8 hours, monitoring for signs of intoxication or mortality, and noted if the bait was regurgitated. If snakes survived for more than 96 hours, checks were reduced to once every 24 hours. Death from acetaminophen toxicity typically occurs within 24 to 48 hours (Savarie et al. 2000; Mathies and Mauldin 2020). Upon presumption of death (cessation of all activity, no perceptible respiration or heartbeat), we monitored snakes for another 4 to 24 hours to verify they were deceased. If the snake survived until the beginning of the following week, we offered another 80 mg ADS bait. If the snake survived the second bait, we offered a third 80 mg ADS bait on the following week. If a snake survived three 80 mg ADS baits, weekly dosing was repeated with a DNM affixed with two 80 mg tablets (160 mg dose) for up to three weeks.

Preliminary results from Phase 1 clearly demonstrated that even repeated weekly exposures to the standard 80 mg ADS baits did not reliably dispatch extremely large snakes. Because 1) much of the interior space of an ADS cartridge is taken by the capsule and ribbon that suspend the very small DNM in the canopy, 2) larger baits might be more attractive to larger snakes; and 3) larger snakes forage on the ground more frequently (Rodda and Reed 2007; Siers 2015; Nafus et al. 2020), we determined that a simple potential solution might be to modify a subset of the bait cartridges by removing the capsule and ribbon components, replacing them with the largest mouse that will fit into the exterior cardboard tube (13–17 g dead "weanling" mice with tail and hind limbs removed), and inserting two 80 mg tablets through the oral cavity with forceps, for a larger 160 mg acetaminophen mouse bait (hereafter "160 mg alternative ground bait"; Figure 2, right). To evaluate the efficacy of these alternative baits, we replicated Phase 1 procedures with minor modifications. During Phase 1, no intervention because of severe signs of pain or distress was required, so our animal health checks for Phase 2 were relaxed to once every 12 hours for up to 96 hours following dosing and every 24 hours thereafter. Each snake that survived dosing with a 160 mg alternative ground bait was offered a second one a week later, and a third one the following week, if necessary.

Statistical analyses

The current study's sample of very large snakes (QA-2927) augmented existing data sets on the efficacy of acetaminophen tablets in DNM baits for brown treesnake control. Our further analyses also include data

collected under WS-NWRC research protocol QA-636, on which the original EPA registration of "Acetaminophen for Brown Treesnake Control" was based (Savarie 2002). These data included snakes ranging in body mass from 29 to 320 g (N = 94) and dosed with uncoated tablets containing 10, 20, 40, 80, and 325 mg of acetaminophen inserted into DNM. We also included data from a USGS/USDA cooperative study (QA-2768) that evaluated differences in mortality between test groups receiving registered 80 mg tablets placed internally or externally (glued) on DNM (Nafus 2021); all snakes in this study weighed between 64 and 279 g body mass, except for two large males that weighed 734 and 760 g (N = 118).

We represent the results of Phase 1 and Phase 2 dosing trials graphically as timelines of survival and mortality. For Phase 1, we performed three logistic regressions, each with ln(snake mass) as the sole predictor of mortality (natural logarithm). Including data from QA-636, QA-2768, and Phase 1 of this study (80 mg doses only), we modeled mortality curves for: 1) all snakes that received a single dose; 2) all snakes that received two or fewer doses spaced approximately one week apart (i.e. including snakes that died after the first dose); and 3) all snakes that received three or fewer doses spaced one week apart (including snakes that died after the first or second doses). We plotted mortality curves against the ln(mass) distribution of the reference population for a heuristic assessment of the proportion of snakes that would be removed after taking one, two, or three doses spaced one week apart. Too few Phase 1 and Phase 2 snakes survived 160 mg acetaminophen dosing for statistical tests, so these results are represented only in the timelines.

Meta-analysis of all dosing data

We pooled acetaminophen tablet dosing data from QA-636, QA-2768, and Phases 1 and 2 of this current study (QA-2927) for a more complete analysis of the efficacy of acetaminophen for lethal control of all brown treesnake sizes. Our primary interest was a full dose-response curve, via logistic regression, incorporating all appropriate data, with dose as the primary predictor. We back-transformed model predictions to a range of LD (lethal dose) values; e.g., LD_{90} is the dose at which 90% of brown treesnakes are predicted to die.

We also sought to determine whether snake sex, body condition, and regurgitation of the bait influenced probability of survival. To avoid uncertainty from confounded methods and repeated dosing, we restricted evaluation of these potential predictors to first doses of 80 mg (N = 218). Separate logistic regressions were employed with each candidate variable + dose as predictors of mortality. Body condition (variation in body mass after controlling for body length) is often used as a proxy for energy stores, overall snake health, and reproductive potential (e.g. Naulleau and Bonnet

1996; Falk et al. 2017), and is occasionally an influential predictor of brown treesnake behavior and response to control tools (Tyrrell et al. 2009; Christy et al. 2010; Clark et al. 2018; Yackel Adams et al. 2019; Nafus et al. 2020). We calculated a condition index (CI) as the ratio of observed to expected snake mass, with expected mass predicted from a third-order polynomial regression of ln(length) as a predictor of ln(mass) for all snakes in the data set. We eliminated one snake with CI > 2.0 (more than twice the predicted mass) and one with CI < 0.5 (less than half of the predicted mass) as these values likely indicated either measurement error or that the snake was in poor health.

Time to death

We also modeled and plotted time until death as a function of dosage. Time to death for QA-2927 snakes (current study) was calculated in hours as the midpoint of the interval between the time the snake was observed to be dead and the previous animal check. For QA-636 and QA-2768, time to death was recorded only in days post-dosing; these were converted to hours, with time of death estimated as 12 hours after the last check when the snake was alive. Modeling was performed with a Cox proportional hazard survival model, with dosage as a predictor variable. Data were restricted to times to death of less than 168 hours (one week) as most snakes that survived for more than one week received another dose.

All statistical tests and data visualization were performed in the R environment for statistical computing, Version 3.5.3 (R Core Team 2019). In keeping with evolving conventions for statistical interpretation, we do not set an arbitrary value of significance for P-values but rather evaluate them as a metric of how compatible the data are with the hypothesis being tested (i.e., very low P-values indicate that the data are highly compatible with the hypothesis; Amrhein et al. 2019).

Results

The large brown treesnakes that we collected and dosed for this study greatly improved representation of the larger end of the range of snake masses, augmenting previous acetaminophen dosing studies (Figure 3). Our combined data set better represents the heavier end of the reference population, our proxy for the size distributions of snakes on Guam. In total, the pooled data set comprises 110 female snakes ranging from 619 to 1,397 mm SVL and 29 to 525 g body mass, and 182 males from 710 to 1,940 mm and 39 to 1,734 g.

Phase 1 dosing trials demonstrated that a single 80 mg ADS bait did not reliably dispatch snakes over 200 g body mass (Figure 4). The lightest female to survive the first 80 mg ADS bait was 245 g, and the lightest male tested in this phase (262 g) also survived the first dose. No female snake survived three weekly 80 mg ADS baits, while males began to survive three 80 mg

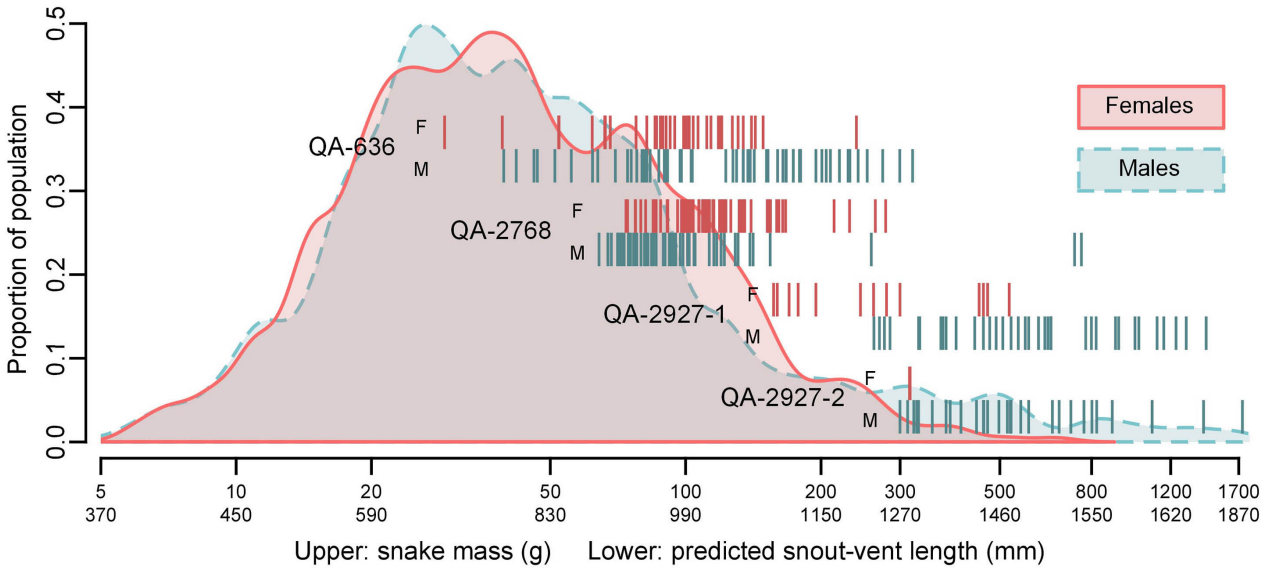

Figure 3. Masses of snakes included in respective data sets (tick marks) plotted over body mass distributions (shaded) for male and female snakes in the reference population. The x-axis is log-distributed, with labels back-transformed into original units. Masses (upper x-axis labels) are actual values, while snout-vent lengths (lower x-axis labels) are predicted average lengths for a given mass based on a length by mass regression.

doses at weights of 374 g and above. Due to human error, four male snakes received only two 80 mg ADS baits before being treated with a 160 mg DNM bait. Only four males, all ≥ 530 g, survived their first 160 mg DNM bait, and no snakes survived a second 160 mg dose. Snakes rarely refused subsequent baits following survival of previous doses. In only one case did a snake refuse a subsequent bait for three weeks, triggering euthanasia per our protocol, though this was the largest snake in the Phase 1 data set (1,440 g).

Logistic regression of mortality by mass with a single 80 mg ADS bait indicates that efficacy starts to deviate from 100% mortality at approximately 150 g of snake body mass, decreasing to 80% mortality around 300 g body mass and 50% at 400 g (Figure 5). By about 650 g of snake mass, a single 80 mg ADS bait is only 20% effective, and by 1,200 g body mass there is virtually no probability of mortality. If surviving snakes take a second 80 mg ADS bait one week later, the cumulative efficacy is improved with 100% mortality extended to approximately 300 g body mass, 50% mortality at 700 g body mass, and approximately 5% mortality at the upper limit of our estimation range (approximately 1,700 g body mass). Marginal improvement is achieved with a third weekly dose of 80 mg ADS bait; by about 1,000 g body mass, three weekly doses is only 50% likely to cause mortality. P-values for a ln(mass) effect on all three curves were < 0.001.

In Phase 2, only two females met the size threshold of 300 g body mass for testing (315 and 316 g). Males in the Phase 2 test group ranged from 300 to 1,734 g. With the exception of the three largest male snakes, all Phase 2 snakes died shortly after receiving their first 160 mg alternative ground bait (Figure 6); all three survivors accepted a second bait one week later, and died shortly thereafter.

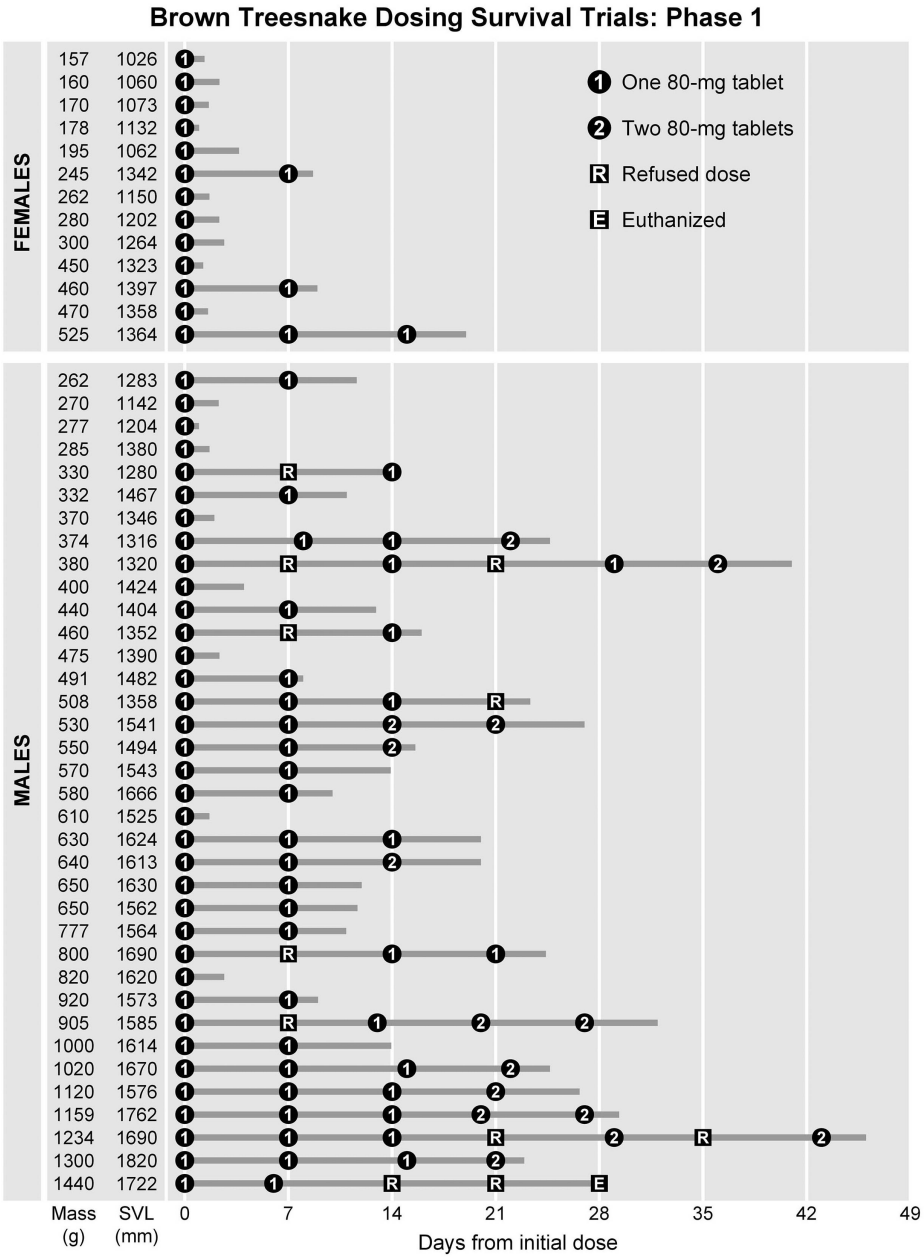

Figure 4. Phase 1 dosing timeline. Baits were 4–6 g dead neonatal mice with one or two 80 mg acetaminophen tablets adhered externally, simulating aerial delivery system baits (Figure 2, left). Horizontal lines terminate at time of death. "Mass" is snake body mass and "SVL" is the snout-vent length of the snake.

Meta-analysis of dosing data

Via logistic regression on the entire data set from all three studies (first DNM bait doses only), we predicted a range of LD values of interest for uncoated acetaminophen tablets within or affixed to a mouse bait (Figure 7): LD_{50} = 167 mg acetaminophen/kg snake body mass; LD_{75} = 224 mg/kg; LD_{90} = 302 mg/kg; and LD_{99} = 572 mg/kg. For eradication the value of interest is generally the LD_{100}, but by the nature of the asymptotic shape of the logistic regression curve, 1.0 (100%) is never reached. The highest dose observed to have been survived was 608 mg/kg, though this appears to be an outlier because the next highest dose survived was 327 mg/kg; 62 snakes

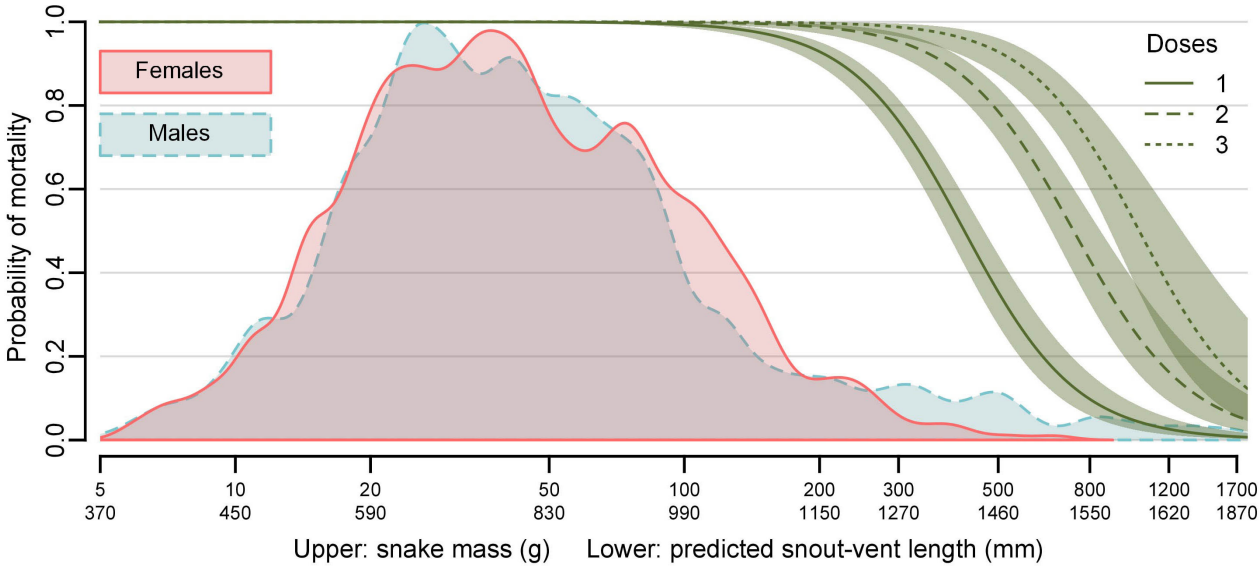

Figure 5. Logistic regression mortality curves for snakes receiving one, two, or three 80 mg acetaminophen doses in dead neonatal mouse baits at one-week intervals. Curves are cumulative; data include all snakes that received the indicated number of doses or died after a previous dose. Shaded areas around logistic response curves indicate ± 1 standard error of the estimate. Background density plots (shaded) represent body mass distributions for male and female snakes in the reference population. The x-axis is log-distributed, with labels back-transformed into original units. Body masses (upper x-axis labels) are actual values, while snout-vent lengths (lower x-axis labels) are predicted average lengths for a given body mass based on a length by mass regression.

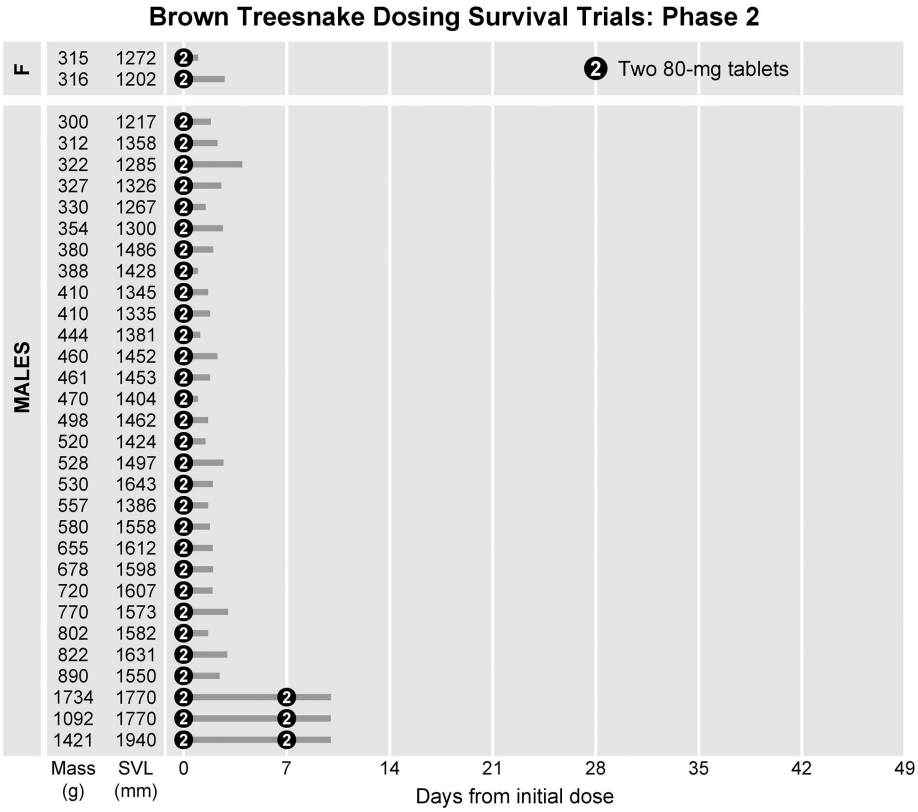

Figure 6. Phase 2 dosing timeline. Baits were 13–17 g dead weanling mice with two 80 mg acetaminophen tablets inserted into the body cavity (Figure 2, right). Horizontal lines terminate at time of death. "Mass" is snake body mass and "SVL" is the snout-vent length of the snake.

treated with intermediate dose levels all died. The lowest lethal dose (LLD or LD_{LO}) observed in our data set was 98 mg/kg which is above the predicted LD_{10}; only 11 snakes in our data set were exposed to dose levels

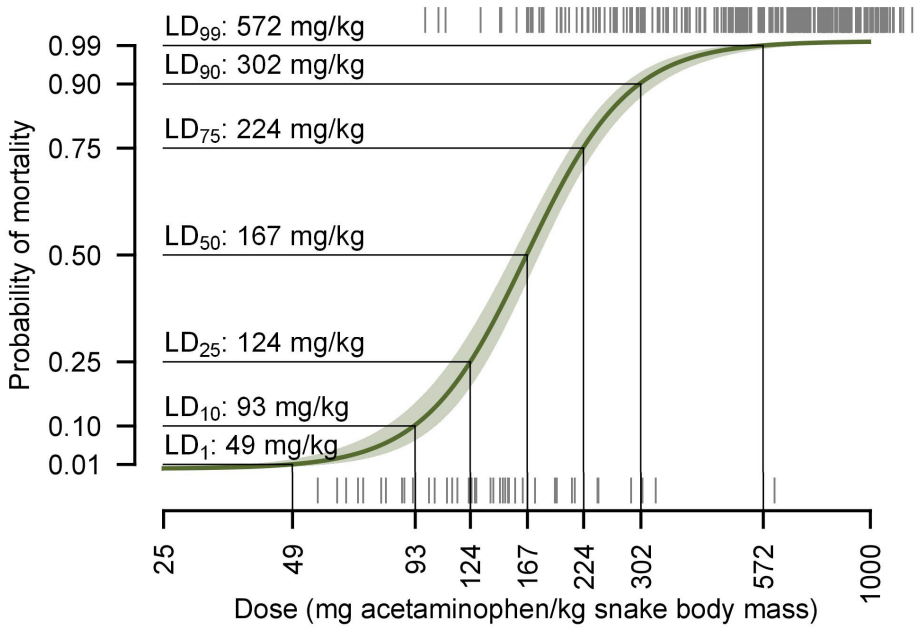

Figure 7. Logistic regression dose-response curve for all first-dose treatments. The shaded area around the curve is ± 1 standard error. LD = lethal dose; e.g., LD_{90} is the dose at which 90% are predicted to die. Gray ticks above and below the curve are doses at which snakes died or survived, respectively.

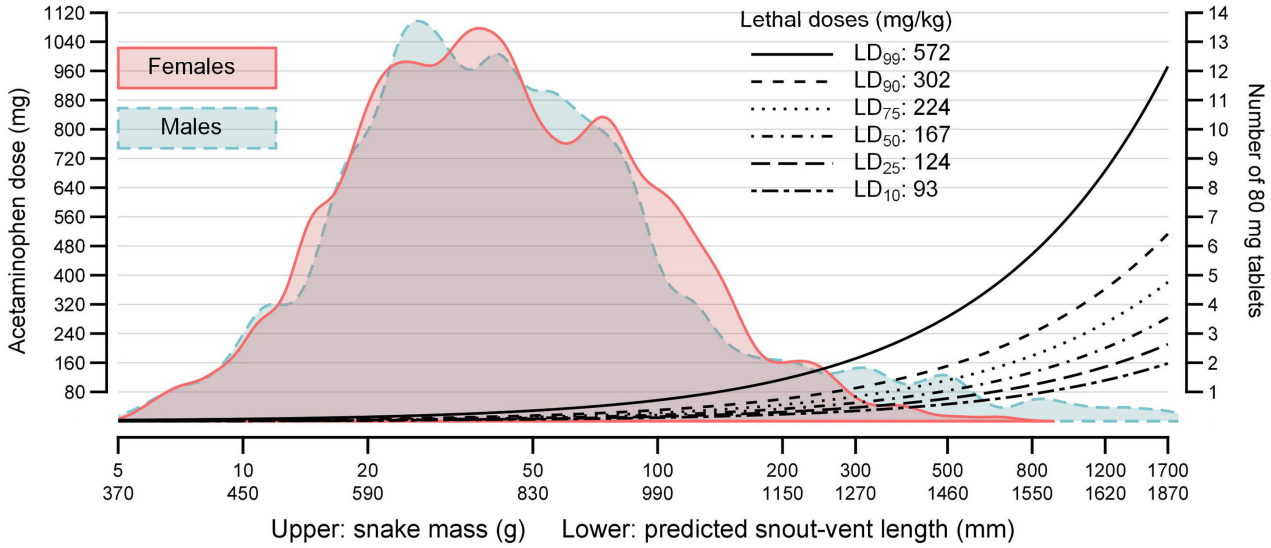

Figure 8. Predicted dose (left y-axis) and number of 80 mg tablets (right y-axis) required to achieve lethal dose (LD) thresholds for removal of snakes on Guam. Background density plots (shaded) represent body mass distributions for male and female snakes in the reference population. The x-axis is log-distributed, with labels back-transformed into original units. Body masses (upper x-axis labels) are actual values, while snout-vent lengths (lower x-axis labels) are predicted average lengths for a given body mass based on a length by mass regression.

below 98 mg/kg, indicating that laboratory efficacy data for severely underdosed snakes is still sparse.

Projecting these data onto the reference population (Figure 8), it is apparent that confident elimination of the largest snakes will require significantly larger acetaminophen doses to be delivered per bait, or for individuals to consume multiple toxic baits in a relatively short period of time. The standard 80 mg dose meets or exceeds the LD_{90} for 95.7% of the reference

Table 2. Percentages of the reference population within the estimated LD_{90} of 302 mg acetaminophen/kg snake body mass that would die from one or two 80 mg acetaminophen tablets (≤ 265 g or ≤ 530 g body masses, respectively). LD_{90} is the dose at which 90% of treated snakes are expected to die. N = sample sizes of each sex (F, M) within the reference population.

Land cover	N	80 mg (≤ 265 g)		160 mg (≤ 530 g)	
		Females	Males	Females	Males
Forest	541 F, 666 M	99.8%	96.7%	100%	98.6%
Savanna	132 F, 172 M	96.2%	90.1%	100%	96.5%
Urban	146 F, 157 M	95.9%	82.8%	99.3%	93.0%
Total	819 F, 995 M	98.5%	93.4%	99.9%	97.4%
Both sexes	1,814	95.7%		98.5%	

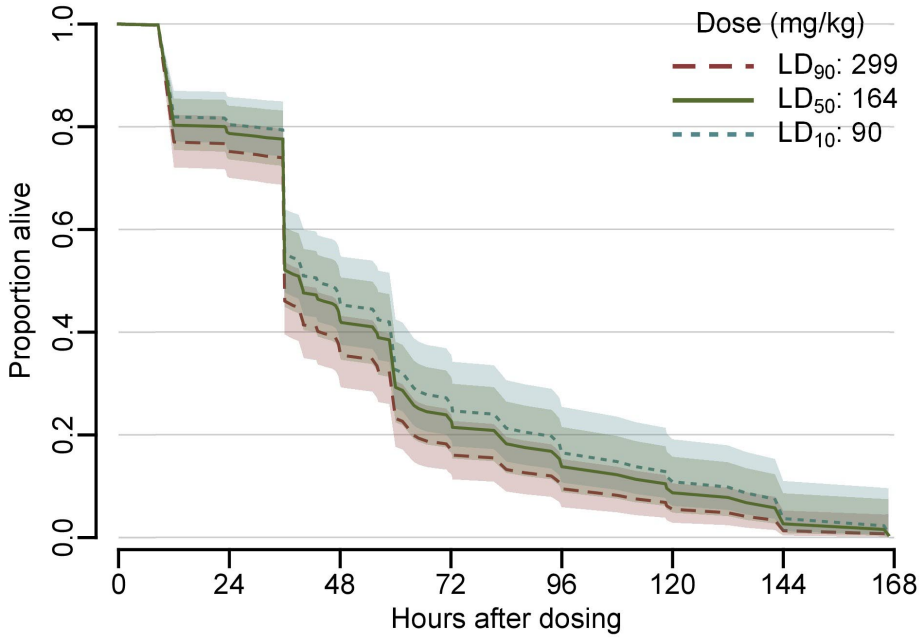

Figure 9. Survival times (time to death) for the subset of brown treesnakes that died at three lethal dose (LD) levels; e.g., LD90 is the dose at which 90% are predicted to die. Shaded areas are ± 1 standard error.

population, and baits with two tablets (160 mg) would meet the LD_{90} for 98.5% (Table 2). Very large females are currently rare in forested areas that the ADS was designed to treat; 80 mg or 160 mg acetaminophen baits would meet the LD_{90} for 99.8% or 100% for forest female snakes, respectively.

After controlling for dose, sex was not a predictor of mortality (P = 0.825). Our data were moderately compatible with a hypothesis of body condition influence on mortality (P = 0.041), with snakes in better body condition being slightly more likely to succumb. We were unable to model an effect of regurgitation, because 100% of snakes that regurgitated baits succumbed to acetaminophen intoxication (77 instances of regurgitation in 346 recorded trials). Most lethally dosed snakes died within 48 hours of dosing (Figure 9). Time to death was influenced by dose (P < 0.001), with snakes receiving higher doses dying more quickly. The most common outward sign of intoxication was ataxia, though this was rarely observed, and severe signs of intoxication (e.g., convulsions, hemorrhaging, hyperactivity) were not observed.

Discussion

We continue to consider acetaminophen an extremely effective toxicant for brown treesnake control, as a single 80 mg dose administered in a mouse bait has proven to be lethal to almost all snakes under 200 g, which are representative of the vast majority of snakes on Guam (Figure 5). The results of this study indicate that consumption of a second or third 80 mg ADS bait a week later will remove even larger snakes. We do not know whether the probabilities of dying on a successive dose are linked to the previous dose(s), i.e., whether lingering effects of the previous dose make snakes more susceptible to a supplementary dose or if mortality on a subsequent dose is simply the result of multiple independent probabilistic events. ADS treatments to date have comprised multiple applications of 120 baits/ha, far exceeding the density of snakes in the habitat (Siers et al. 2020b). DNM baits typically remain viable in the field for 48 to 72 hours (Siers et al. 2019) and multiple takes of nontoxic bait by individual transponder-tagged snakes at multiple bait stations in a single night has been documented (Campbell and Sugihara 2001), so it is possible that individuals could encounter and consume multiple doses within a short window of time, greatly increasing the probability of removing very large snakes with existing ADS baits. Although conditioned taste aversion has been demonstrated in other reptiles (e.g., Ward-Fear et al. 2017), our results indicate very little reluctance for brown treesnakes to accept another treated bait one week after surviving a prior intoxication, which also bodes well for our ability to effectively remove a snake that survived a previous acetaminophen bait.

A doubling of the number of EPA-registered 80 mg acetaminophen tablets would double the range of body masses of snakes at risk of lethal intoxication. Coupled with a slightly larger bait matrix that could be incorporated into the existing ADS (Figure 2, right), to be delivered to the ground where larger snakes forage more frequently, these alternative ground baits could increase the effectiveness of ADS for larger, more fecund snakes that pose greater threat to humans, domestic animals, and wildlife. In a pilot test in a closed and marked brown treesnake population undergoing simulated ADS treatments, supplementing the standard 80 mg ADS baits with 13–17 g mouse baits with a single internal 80 mg tablet, placed on the ground, demonstrated an increase in effectiveness for snakes > 1150 mm SVL (Nafus et al. 2020b); coupled with higher doses (i.e. two tablets), efficacy of ADS for very large snakes could be further improved by incorporating such baits. Fortunately, the range of snake sizes for which 80 or 160 mg doses are effective include the vast majority of females on Guam, particularly in forest habitats where very large snakes are currently rare (Table 2).

Our results indicate that extraordinarily large snakes, primarily males > 750 g, will require more than a single 160 mg dose to be effectively removed. These snakes are extremely rare, particularly in forested areas where large prey are scarce due to brown treesnake predation (Siers 2015; Siers et al. 2017). Prey availability would be expected to increase following snake suppression and reintroduction of native vertebrates, however, and this would be anticipated to cause an increase in abundance of snakes in larger size classes. Effectively removing large brown treesnakes is an important goal for pest management to support native species recovery on Guam. It is possible that a small number of much larger baits—too large to be taken by average brown treesnakes, containing far greater doses of acetaminophen—may be helpful in removing very large males; however, bait matrices comprised of dead animals degrade very quickly and the probability of large snakes encountering a small number of very large baits on the landscape is likely to be quite low. Also, nontarget species risks posed by baits with high acetaminophen doses may be unacceptable, as crabs and carnivorous lizards are abundant on the forest floor and feral and domestic animals occur in forests closer to residential and urban areas (Johnston et al. 2002). Moreover, if reintroduced, several native birds would likely scavenge mouse bait, and bait presence in the canopy or on the forest floor could result in avian mortality. It is likely that control of very large snakes may be limited to trapping in urban areas where they are more frequently found, though existing trap and lure systems are not designed with very large snakes in mind. Development of traps or other control tools for very large snakes remains an area that requires further advancement.

Like acetaminophen, other common drugs have been demonstrated as effective vertebrate pesticides (Eason et al. 2014, 2017; Shapiro et al. 2015; Eason 2018). To our knowledge, this is the first thoroughly documented dose-response curve for an invasive vertebrate with indeterminate growth. Challenges for chemical control of the largest individuals include selectively targeting extremely rare large individuals with baits containing a much larger toxicant dose without posing undue risk to nontargets. Even the largest brown treesnakes are relatively small compared to some invasive reptiles for which acetaminophen has been considered as an oral toxicant, such as invasive Argentine giant tegu (*Salvator merianae*, up to 8 kg body mass), Nile monitors (*Varanus niloticus*, up to 20 kg), and Burmese pythons (*Python molurus bivittatus,* up to 90 kg) (Enge et al. 2004; Hardin 2007; Mauldin and Savarie 2010; Dorcas et al. 2012). Effectively and safely targeting the largest individuals of brown treesnakes and other species with indeterminate growth, given the very wide range of body sizes, is likely to remain a primary challenge for management or eradication of these harmful invasive predators.

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Ethics Approval

Animal care and use approvals and environmental compliance are documented in USDA approved research protocol QA-2927.

Data Availability

USDA data are available on request from the corresponding author or the archives of the Quality Assurance Unit of the USDA Wildlife Services National Wildlife Research Center. USGS data are available for download at https://doi.org/10/5066/P9HJIBE8 and https://doi.org/10.5066/P9WCZW5V.

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