

Research Article**Acetaminophen as an oral toxicant for invasive California kingsnakes (*Lampropeltis californiae*) on Gran Canaria, Canary Islands, Spain**Jake Friebohle^{1,*}, Shane R. Siers² and Chad E. Montgomery¹¹Department of Biology, Truman State University, Kirksville, MO 63501, USA²U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Hilo, HI 96720, USAAuthor e-mails: jakeafriebohle@gmail.com (JF), shane.r.siers@usda.gov (SRS), chadmонт@truman.edu (CEM)

*Corresponding author

Citation: Friebohle J, Siers SR, Montgomery CE (2020) Acetaminophen as an oral toxicant for invasive California kingsnakes (*Lampropeltis californiae*) on Gran Canaria, Canary Islands, Spain. *Management of Biological Invasions* 11(1): 122–138, <https://doi.org/10.3391/mbi.2020.11.1.09>

Received: 31 July 2019**Accepted:** 1 November 2019**Published:** 31 January 2020**Handling editor:** Staci Amburgey**Thematic editor:** Catherine Jarnevich**Copyright:** © Friebohle et al.

This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International - CC BY 4.0).

OPEN ACCESS**Abstract**

Invasive species are threatening biodiversity and ecosystem stability globally. The introduction of the California kingsnake (*Lampropeltis californiae*) on Gran Canaria, Canary Islands, Spain represents an emerging invasion that is already threatening endemic island species. Dead neonatal mice treated with 80-mg acetaminophen tablets are approved as a registered pesticide for control of invasive brown treesnakes (*Boiga irregularis*) in Guam and could potentially be used as an oral toxicant to control invasive California kingsnake populations. We sought to evaluate oral toxicity of acetaminophen and to determine the dosage necessary for lethal control of invasive California kingsnake populations. Dead mice inserted with a known acetaminophen dose (0 mg, 40 mg, 60 mg, and 80 mg) were fed to California kingsnakes from Gran Canaria. Each dose was tested in 20 male and 20 female snakes representing the size range found in Gran Canaria. After snakes ate their dead mouse, they were monitored for mortality, regurgitation, and time of death and regurgitation. Treatments of 60 mg and 80 mg had 100% mortality, while 40 mg had 87.5% mortality. No control snakes died. Time to death occurred on average 38.6 hours after consuming the dead mouse. The top two time to death models accounted for 97% of model weights and included variables dosage (mg/kg), sex, and dosage * sex or those terms plus body condition index. Out of the 116 snakes that died, 97 regurgitated the mouse that contained the acetaminophen capsule, and time to regurgitation was highly correlated with time to death. Acetaminophen is a highly effective oral toxicant for California kingsnakes. Dead mouse baits treated with acetaminophen have potential as a control method on Gran Canaria but should not solely be expected to protect native species or eradicate California kingsnakes on Gran Canaria. Future efforts should focus on preventing California kingsnakes from invading other Canary Islands.

Key words: endemic species, island conservation, population control, snakes**Introduction**

Many reptiles have successfully invaded ecosystems outside of their natural range (Kraus 2009). For example, the Balearic Islands have more invasive than native reptiles (Pinya and Carretero 2011). Invasive reptiles are often generalist (wide diet breadth and climate tolerance), which aids them in invading ecosystems with environmental conditions that overlap with

those of their native ranges (Filz et al. 2018). When reptiles are introduced intentionally (e.g. releasing unwanted pets) or accidentally (e.g. stowaways in cargo) into novel ecosystems, they can have negative impacts on native species. For instance, the brown anole (*Anolis sagrei* Duméril and Bibron, 1837) has successfully invaded the southeastern United States from the Bahamas and displaced the native green anole (*Anolis carolinensis* Cuvier and Voigt, 1832) to fringe habitat throughout much of its range (Stuart et al. 2014), and the invasive common house gecko (*Hemidactylus frenatus* Schlegel, 1836) has outcompeted native species, such as the mourning gecko (*Lepidodactylus lugubris* Duméril and Bibron, 1836), across the Pacific basin for food resources (Petren and Case 1996).

Invasive snake populations are threatening biodiversity of island and mainland ecosystems because snakes are effective predators (Campbell et al. 2012). Reducing an invasive snake population's threat towards native species is challenging because snakes are difficult to locate and remove due to their cryptic nature. International trade and human movement have established multiple invasive snake populations. Transporting ornamental plants, primarily olive trees, is thought to be a main vector of recent establishment of invasive horseshoe whip snake (*Hemorrhois hippocrepis* Linnaeus, 1758), montpellier snake (*Malpolon monspessulanus* Hermann, 1804), and ladder snake (*Rhinechis scalaris* Schinz, 1822) populations in the Balearic Islands (Silva-Rocha et al. 2015). The introduction of the wolf snake (*Lycodon aulicus* Linnaeus, 1758) in Christmas Island, Australia (Fritts 1993) and Mauritius (Tonge 1990) and the brown treesnake (*Boiga irregularis* Bechstein, 1802) in Guam (Rodda et al. 1992) are also thought to be due to snakes becoming stowaways in ships' cargo. Other invasive snake populations have been aided by the exotic pet trade. Pet snakes being released or escaping captivity have established invasive populations of Burmese pythons (*Python molurus* Linnaeus, 1758) and boa constrictors (*Boa constrictor* Linnaeus, 1758) in southern Florida (Snow et al. 2007; Dorcas et al. 2012) and corn snakes (*Elaphe guttata* Linnaeus, 1766) and boa constrictors in multiple Caribbean islands (Perry et al. 2003; Bushar et al. 2015).

Invasive snake populations can potentially have large cascading effects on ecosystems they invade. The invasion of the Burmese python into southern Florida has resulted in the dramatic decline of the medium-sized mammal community (Dorcas et al. 2012). Dramatic population reduction (87.5–100% loss) in raccoons (*Procyon lotor* Linnaeus, 1758), Virginia opossums (*Didelphis virginiana* Kerr, 1792), marsh rabbits (*Sylvilagus palustris* Bachman, 1837), eastern cottontails (*Sylvilagus floridanus* J.A. Allen, 1890), gray foxes (*Urocyon cinereoargenteus* Schreder, 1775), red foxes (*Vulpes vulpes* Linnaeus, 1758), bobcats (*Lynx rufus* Schreber, 1777), and white-tailed deer (*Odocoileus virginianus* Zimmermann, 1780) have occurred since the establishment of the Burmese python in Everglades National Park (Dorcas et al. 2012). One of the largest ecological and economic catastrophes due to

a reptilian invasive species is the invasion of the brown treesnake on Guam, which resulted in the loss of native birds (Savidge 1987; Wiles et al. 2003), mammals (Rodda et al. 1997), and lizards (Rodda and Fritts 1992; Campbell et al. 2012) through overconsumption. The collapse of Guam's native avifauna has resulted in an ecosystem cascade affecting other taxa, such as arthropod distribution and abundance (Rogers et al. 2012) and loss of ecosystem services such as seed dispersal (Rogers et al. 2017).

An emerging snake invasion is occurring on Gran Canaria, Canary Islands, Spain, with the introduction of the California kingsnake (*Lampropeltis californiae* Blainville, 1835), a medium-sized constricting colubrid. The California kingsnake is a generalist predator that is widely distributed in western and southwestern United States and northwestern Mexico (Markel 1989; Stebbins 1998). Since 1998, California kingsnakes have been found outside of captivity on Gran Canaria and been considered officially established in 2007 (Pether and Mateo 2007). California kingsnakes were introduced to Gran Canaria through the pet trade, and either escaped or were released from captivity into an ecosystem with no natural predators. Currently there are established California kingsnake populations in Telde-Valsequillo, Gáldar, and Maspalomas in Gran Canaria (Fisher et al. 2019). The established populations are genetically distinct, indicating that they are from different founder groups (Monzón-Argüello et al. 2015).

There are no native snake species on Gran Canaria, so California kingsnakes represent a novel threat to prey species. In areas of Gran Canaria inhabited by California kingsnakes, endemic lizard populations are at risk, including Gran Canaria skink (*Chalcides sexlineatus* Steindachner, 1891) and Gran Canaria giant lizard (*Gallotia stehlini* Schenkel, 1901) due to California kingsnake predation (Cabrera-Pérez et al. 2012; Monzón-Argüello et al. 2015). Size distribution of Gran Canaria giant lizard populations have shifted towards primarily larger adults that are too big for California kingsnakes to consume (Gallo-Barneto R, GESPLAN, *unpublished*). The decreasing Gran Canaria giant lizard population could impact local vegetation because Gran Canaria giant lizards are frugivores and potentially important seed dispersers like the closely related *Gallotia galloti* Oudart, 1839 on nearby Tenerife (Valido et al. 2003).

Gran Canaria has contracted Gestión y Planeamiento Territorial y Medioambiental S.A. (GESPLAN), a private organization, to manage LIFE+ Lampropeltis, a California kingsnake capture and public outreach program. LIFE+ Lampropeltis has utilized active searching, funnel traps, coverboard traps, and a public call response program to attempt to capture California kingsnakes to achieve their primary goal of reduction and eventual eradication of California kingsnake populations on Gran Canaria. LIFE+ Lampropeltis's capture methods have not been able to stop California kingsnake's continual range expansion. Gran Canaria California kingsnakes have greater average clutch size (Cabrera-Pérez et al. 2012) and

body mass index (Fisher et al. 2019) compared to California kingsnakes in their native range. Due to ample food resources and minimal predation, California kingsnakes have been able to rapidly increase population size and have the potential to continue to do so. Additional methods are needed to prevent further California kingsnake spread on Gran Canaria and increased endangerment of endemic species.

Following the invasion of Guam by accidentally-introduced brown treesnakes, and the subsequent ecological and economic impacts (Savidge 1987; Wiles et al. 2003; Rodda and Savidge 2007), acetaminophen (known internationally as paracetamol) was identified as a safe, humane, and effective toxicant for brown treesnake control (Savarie et al. 2000; Johnston et al. 2002). Acetaminophen mouse baits—80-mg tablets coupled with a dead newborn mouse (DNM) bait, the most effective brown treesnake bait matrix tested to date (Savarie and Clark 2006)—have become an integral part of brown treesnake interdiction and control programs on Guam (Clark et al. 2018) and are registered for use as a brown treesnake pesticide with the US Environmental Protection Agency (EPA Reg. No. 56228-34). Hand-placing of acetaminophen mouse baits in bait stations can be more cost-effective than trapping (Clark et al. 2012) and has demonstrated the ability to greatly reduce brown treesnake abundance on a landscape scale (Savarie et al. 2001). Acetaminophen mouse baits have also been adapted and proven effective for aerial bait applications over inaccessible forest habitats (Clark and Savarie 2012; Dorr et al. 2016) and are currently being manufactured for distribution via an automated aerial delivery system for landscape-scale control of brown treesnakes (Siers et al. 2019). The first small-scale aerial eradication attempt has commenced in an experimental forest plot surrounded by a snake exclosure fence (SRS, *unpublished*).

Subsequent to demonstrating effectiveness for brown treesnake control, acetaminophen has been tested as a tool for lethal control of other invasive reptiles and been found effective for juvenile Burmese pythons (Mauldin and Savarie 2010), juvenile Nile monitors (*Varanus niloticus* Hasselquist, 1762; Mauldin and Savarie 2010), and Jackson's chameleons (*Trioceros jacksonii* Boulenger, 1896; Van Kleeck and Holland 2018). Results were less promising for black spiny-tailed iguana (*Ctenosaura similis* Gray, 1831; Avery et al. 2011), at least at low dosages. These results have not yet translated to any significant management actions for invasive reptile control.

Lethal control of wildlife is always controversial and invokes concerns about humaneness of methods to be employed. The proximate mode of action causing death by acetaminophen toxicity in brown treesnakes is methemoglobinemia, wherein elevated levels of methemoglobin interfere with blood cells' ability to bind or release oxygen resulting in anemic hypoxia (Mathies and Mauldin 2020). Methemoglobinemia is considered a relatively humane mode of action with low severity and short duration of

symptoms (Ruell et al. 2019), similar to CO₂ immersion which is considered to be a humane means of euthanasia (AVMA 2013).

Although acetaminophen has been evaluated as a humane and effective toxicant for other invasive reptiles, any recommendations for application in a management scenario should be preceded by confirmation of its efficacy for the target species. Given the potential dire consequences of the California kingsnake invasion of the Canary Islands, we sought to evaluate oral toxicity of acetaminophen and to determine the dosage necessary for lethal control of invasive California kingsnake populations.

Materials and methods

Study animals

We obtained a total of 132 California kingsnakes from Gran Canaria for this study. All snakes we used for the study were either captured with funnel or coverboard traps or active searches in Telde-Valsequillo and Gáldar by LIFE+ Lampropeltis. LIFE+ Lampropeltis shipped all snakes to O'Hare International Airport, Chicago in May 2017, where we transported the snakes to their housing facilities at Truman State University in Magruder Hall room 3434, Kirksville, Missouri. For each snake, we determined sex by cloacal probing (Reed and Tucker 2012), measured mass (0.1 g) by placing a snake into a tared container on an electronic balance, and measured snout-vent length (SVL; 0.1 cm) by gently stretching the snake's body along a flexible cloth measuring tape. We assigned each snake a unique individual identification number. We then housed 1 to 5 snakes together based on similar size and same sex in plastic containers (57.9 × 42.4 × 17.8 cm or 34.6 × 21 × 12.4 cm) with newspaper substrate, a hide box, outside label with snake identification number(s), and water ad libitum. Group-housed snakes were separated for feedings to prevent competition. We checked snakes daily to ensure cages were clean and had enough water. We allowed snakes to acclimate for three months at a room temperature of 25 °C before trials began. After the acclimation period, we fed snakes a dead feeder mouse each month until they were tested (Truman State University raised mice equivalent to ~ 10% of the snake's body mass).

Body condition index

We derived a snake body condition index (CI) as the residuals from a quadratic linear regression of log(mass) and log(SVL) and scaled to a standard normal distribution so that values are in units of standard deviations from the mean of zero (e.g., Siers et al. 2017 PLOS ONE). Snakes with positive CI values are heavier than predicted by length and are presumed to have greater energy stores (Bonnet and Naulleau 1995).

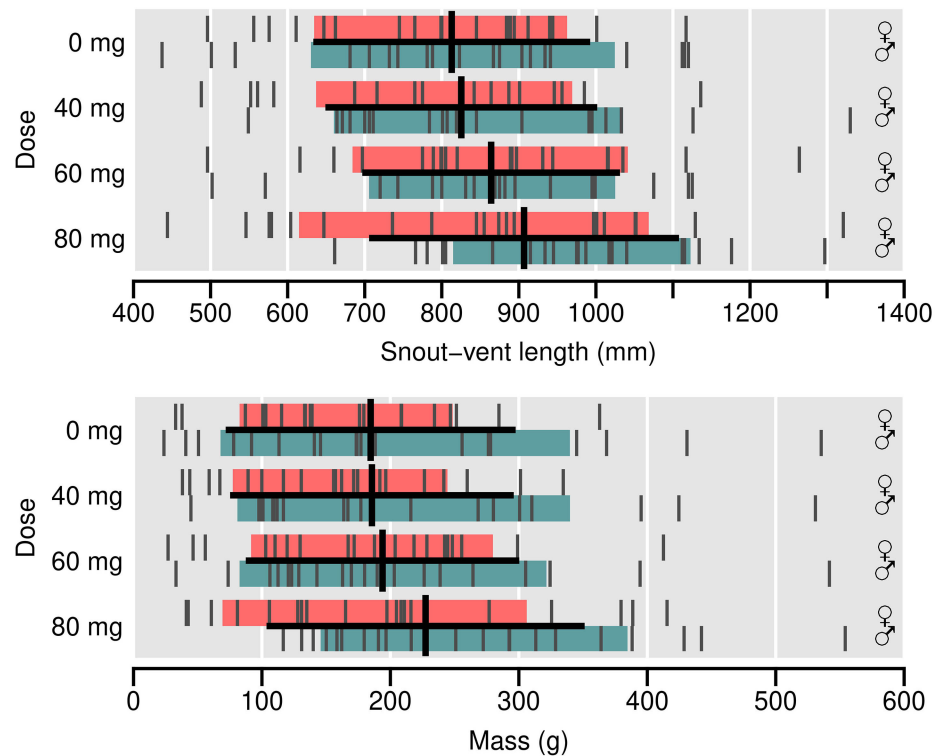


Figure 1. Length and mass distributions for treatment groups. Small vertical ticks are individual values for females (♀, above center line) and males (♂, below). Red and blue boxes are mean $\pm$ 1 standard deviation for females and males, respectively. The dark vertical line is the mean of both sexes, and the dark horizontal line is $\pm$ 1 standard deviation of both sexes. Statistical tests for differences among all pairwise comparisons of treatment groups indicated no substantial length or mass biases.

Test group assignment and treatments

We assigned snakes to treatment groups that would receive acetaminophen doses of 0, 40, 60, and 80 mg [model term DOSE] and derived the mass-specific dosage [DOSAGE] as mg of acetaminophen per kg of snake mass (mg/kg). We assigned snakes to dose-specific treatment groups with equal sex ratios and such that distributions of size and mass would not be biased among groups. We verified that there were no biases in length and mass with an ANOVA and confirmed no pairwise differences among test groups with a Tukey's HSD test. There were no marked length or mass differences in pairwise comparisons among treatment groups (Tukey-adjusted $p = 0.097$ – 0.990 [SVL] and 0.334 – 1.000 [mass]). See Figure 1 for comparison of length and mass distributions.

We prepared acetaminophen treatments by filling gelatin capsules with measured doses of powdered acetaminophen (CAS#103-90-2, Sigma-Aldrich, St. Louis). We implanted capsules into dead feeder mice (3.1–10.0 g) via abdominal incisions; mice for the 0-mg control group were implanted with empty capsules. At the beginning of the trial we offered each snake a treated mouse, which was either voluntarily ingested or we coerced the snake to feed by inserting the mouse into the snake's oral cavity.

Our initial treatment group sizes were $N = 40, 35, 29,$ and $28,$ respectively. After establishing 100% survival in the 0-mg negative control

group, we re-used 29 of the control snakes in the treatment groups to maximize the amount of data that could be obtained with the limited number of animals available for trials. Re-used snakes were selected to balance sex and mass among treatment groups. After we reassigned control group snakes to the 40, 60, and 80-mg treatment groups, our sample sizes were $N = 20:20, 20:20, 20:20$, and $20:21$ (F:M), respectively. In order to control against potential artefacts of re-use of control snakes, we assigned a data covariate to identify individuals that were re-used [CONTROL]. We later evaluated whether re-use of these individuals had any influence on mortality or time until death; if the term carried no explanatory value, we removed it from the model.

We fed snakes between the hours of 1000 and 1400 and checked snakes every 2 hours between 7 a.m. and 11 p.m. until they had died or survived for more than 7 days. We recorded whether the snake had regurgitated its treatment mouse [REGURG], and recorded time to death [TTD] and time to regurgitation [TTR] as the midpoint between successive observations when death or regurgitation were noted (hours). We also noted general observations of snake behavior during the trials. All animal procedures were reviewed and approved by the Truman State University Institutional Animal Care and Use Committee (IACUC) per Protocol #17-7.

Statistical methods

Factors affecting efficacy (percent mortality) would be properly evaluated with a logistic regression, with effect size assessed in the model output. However, as we will report in Results, our data did not have enough variability to be properly modeled. Rather, we estimated and plotted means and 95% binomial compatibility intervals (exact method) and tested all pairwise comparisons among treatment groups using the R function “pairwise.prop.test” (Holm method) for p -value adjustments for multiple comparisons.

For snakes that died, we evaluated factors influencing time to death using Cox proportional hazard models as implemented in the R package “survival” (<https://github.com/therneau/survival>). Because dosage in relation to mass (mg/kg) is derived from dose and mass, and dosage is the more direct measure of the mechanics of toxicity, we elected to only consider models including the term DOSAGE rather than terms for dose and mass. We also considered the influence of terms for sex [SEX], an interaction of sex and dosage [SEX*DOSAGE], body condition index [CI], whether the snake regurgitated [REGURG], and whether the snake had previously been used in the control group [CONTROL]. We log-transformed and scaled all continuous predictor variables for modeling to improve normality of distribution and model convergence [SVL, MASS, CI, DOSAGE]. We evaluated models with all combinations of these terms in an Aikake’s Information Criterion (AIC) model selection framework (e.g., Burnham and

Anderson 2002) adjusted for small sample size (AIC_c). We considered the summed weights of models containing respective terms as an index of relative variable importance (RVI; Anderson 2008). Per convention, we considered models with a ΔAIC_c of -2 or less to be superior models, and models within ΔAIC_c of -2 of the top model to be plausible alternative models. As a check against uninformative parameters (Arnold 2010)—which increase AIC_c by ~ 2 units over the same model without that term while adding nothing to the model fit as evaluated by adjusted R^2 —we also verified compatibility of the data with respective hypotheses based on p -values from z -distributions.

We evaluated the relationship between time to regurgitation and time to death with a linear regression model. Because they were tightly correlated, we do not report Cox proportional hazard models for time to regurgitation; preliminary exploratory modelling showed nearly identical results to time to death models. For each dosed individual that regurgitated and died, we calculated the proportional relationship between times to regurgitation and death (time to regurgitation*time to death⁻¹).

All statistical tests were performed in R version 3.6.0 (R Core Team 2019). In addition to AIC_c values, p -values from statistical tests are evaluated for compatibility of the data with the respective hypothesis without assignment of an arbitrary threshold of significance or non-significance, and 95% compatibility intervals are assessed for the point estimates (*sensu* Amrhein et al. 2019).

Results

Acetaminophen dosing was highly efficacious, with 100% mortality in the 60 and 80-mg treatment groups, and 87.5% mortality (5/40 surviving) in the 40-mg treatment group. In pairwise comparisons of mortality rates, the control group, with zero mortalities, was clearly different than any of the treatment groups ($p < 0.001$). The p -value for the difference between the 40 mg treatment group and the 60 or 80 mg groups (0.121) was marginally compatible with a hypothesis of lower efficacy (Figure 2). When evaluated by sex, results were the same with no differences between males and females within treatment group, and all treated groups differed from the control group ($p < 0.001$).

The dosages we evaluated ranged from 75.3 to 2230 mg/kg (Figure 3). The highest dosage that a snake survived in this study was 401 mg/kg; this appears to be an outlier because all 71 snake dosages in between the highest and second highest survival dosage (133 mg/kg) died (Figure 3). Including this extreme value, our data set would indicate that the LD_{100} (dosage above which all snakes died) is 401 mg/kg, with an LD_{99} (dosage above which 99% of snakes died) of > 133 mg/kg. The lowest lethal dosage ($LDLo$) in this study was 94.2 mg/kg. Because our tests were so efficacious, we did not get enough survival data across a range of lower dosages in order to calculate

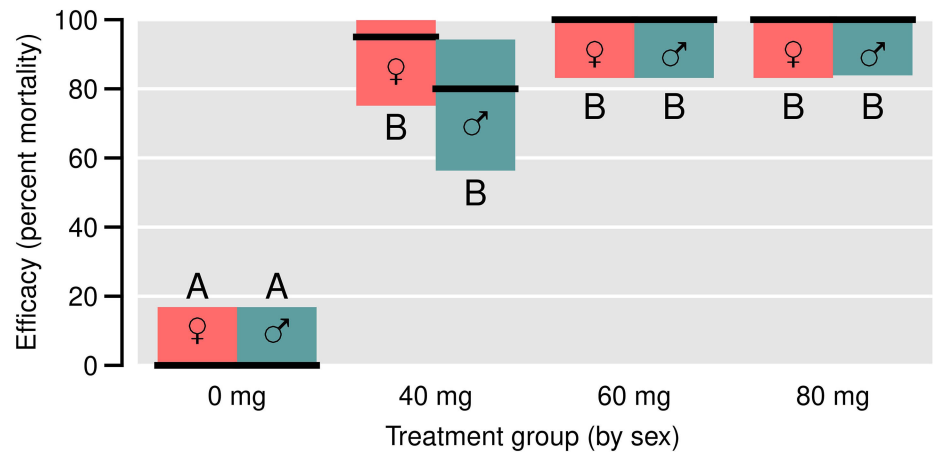


Figure 2. Observed mortality rates by treatment group and sex. Lines indicate point estimates of mortality rate (%) and shaded areas are the 95% compatibility intervals of the estimate.

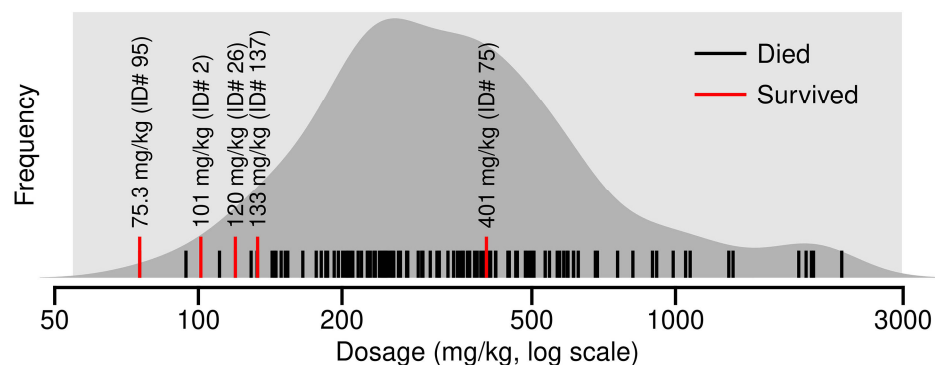


Figure 3. Dosages (mg/kg) evaluated in this study (log scale). Black ticks indicate snakes that died, red indicates survivors. The dark gray area is the frequency distribution of dosages that were evaluated.

Table 1. AICc model selection for factors influencing time to death.

Model	DF	LogLik	AICc	Δ AICc	Weight
DOSAGE+SEX+DOSAGE*SEX+CI	4	-412.6	833.57	0	0.608
DOSAGE+SEX+DOSAGE*SEX	3	-414.1	834.46	0.89	0.389
DOSAGE+SEX+CI	3	-419.6	845.38	11.81	0.002
DOSAGE+SEX	2	-421.6	847.22	13.64	0.001
DOSAGE+ CI	2	-422.3	848.75	15.17	0.000
DOSAGE	1	-423.9	849.86	16.29	0.000
CI	1	-432.0	865.98	32.41	0.000
SEX+CI	2	-431.4	866.81	33.24	0.000
(Intercept only – null model)	0	-438.7	877.43	43.85	0.000
SEX	1	-438.7	879.44	45.87	0.000

a full dosage response curve; however, because our objective was to identify a reliably lethal dose, use of snakes for a lower-dose treatment group would not have been justified.

Time to death

Time to death ranged from 14 to 80 hours after ingestion of acetaminophen-treated mice, with a mean of 38.6 hours (SD $\pm$ 13.2). All models comprising the top 99% of total model weights included the terms DOSAGE, SEX, and DOSAGE*SEX as predictors of time to death. The

model containing only these terms outcompeted the top model that did not include all three by $\Delta AIC_c = -10.92$, and the top model that included none of them by -31.52 . Other models within the top 99% of summed model weights included these terms along with one or more of the remaining terms of CI, REGURG, and CONTROL. However, in the models containing DOSAGE+SEX+DOSAGE*SEX plus just one of these terms, p -values for REGURG and CONTROL were very high, indicating that these were uninformative parameters with no true influence on model fit. After removing these terms, we generated a full model set with the remaining terms (Table 1). The top two models in this set accounted for 97% of model weights and included only DOSAGE+SEX+DOSAGE*SEX or those terms +CI.

Most snakes (70.7%) succumbed between 24 and 48 hours after ingestion of acetaminophen treatments, consistent with results from prior brown treesnake studies (Savarie et al. 2000; Nafus and Siers 2017), and 50% of lethally intoxicated snakes died by 36 hours. DOSAGE was a highly influential predictor of time to death, being included in models carrying $\approx 100\%$ of AICc model weights, with the top model including DOSAGE being 32.41 AICc units lower than the top model without DOSAGE (p -value from the top model = 0.003). Snake sex affected survival times ($p = 0.001$), with females succumbing more slowly than males (Figure 4A). There was a strong interaction between SEX and DOSAGE: males receiving a high dosage succumbed more quickly than females receiving the same dosage, but males receiving a low dosage also succumbed more slowly than females receiving that dosage (Figure 4B). Snakes in poorer body condition succumbed more quickly than snakes that were heavier for their length (Figure 4C), but the effect was modest; this term was included in the top model carrying 68% of model weights, and the z -statistic suggested compatibility with an effect of CI ($p = 0.084$).

Regurgitation

Of the 116 dosed snakes that died, 97 (83.6%) regurgitated the mouse that contained the acetaminophen capsule. Time to regurgitation ranged from 9.7 to 80 hours after ingestion, with a mean time of 27.5 hours ($SD \pm 10.8$). Time to regurgitation was highly correlated with time to death (F -statistic = 197.39, $p \ll 0.001$, adjusted $R^2 = 0.672$). On average, regurgitation occurred at 71.5% of the time interval between ingestion and death ($SD \pm 14.7\%$, range = 23 to 100%). In contrast, only 2/40 (5%) of snakes in the 0-mg treatment group regurgitated.

General Observations

After consuming a treatment mouse, snakes generally did not move from the corner of their container. Snakes that died were lethargic and unable to right themselves when flipped over onto their backs. Several snakes were

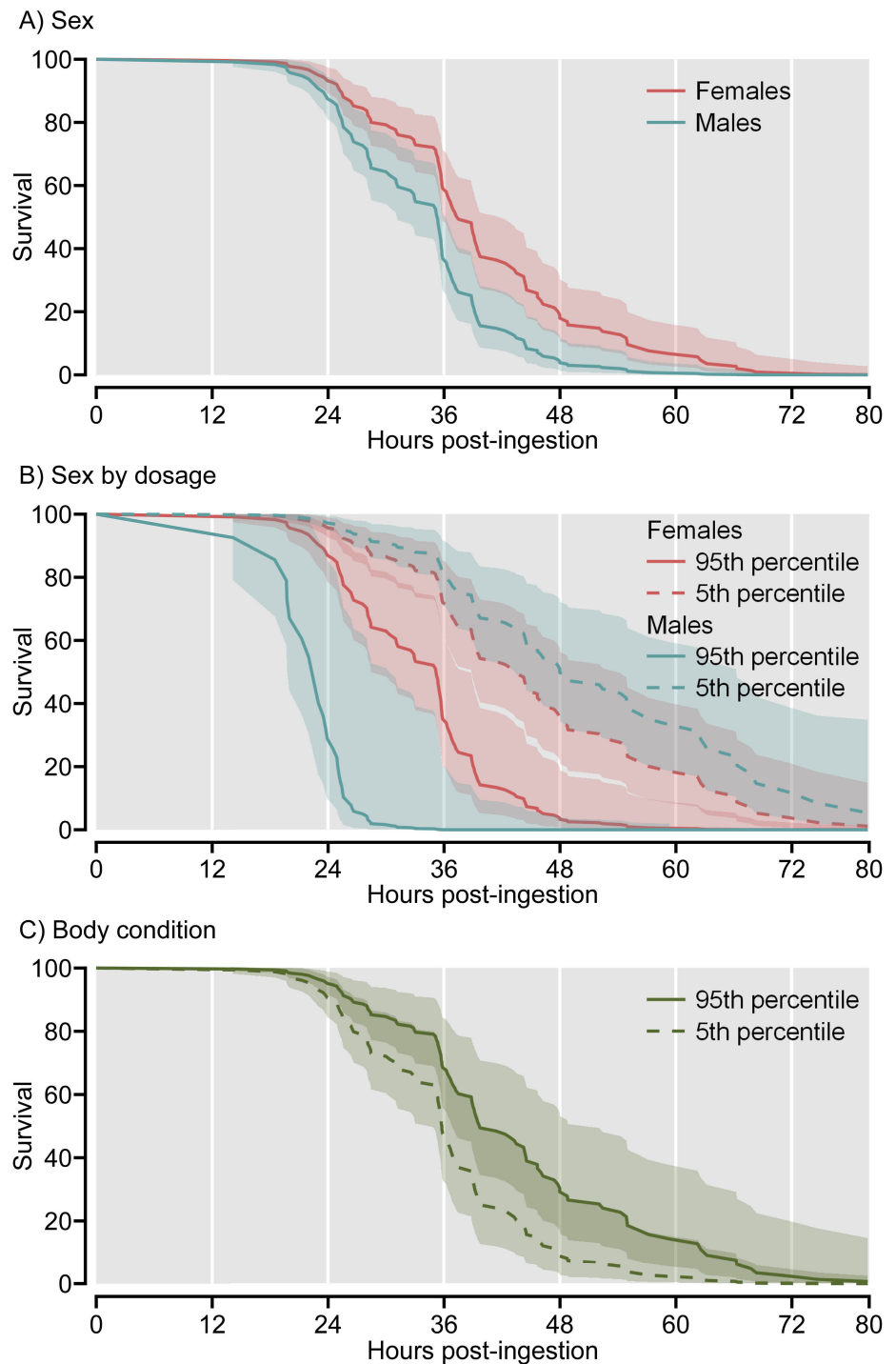


Figure 4. Survival curves for time to death of all dosed California kingsnakes that succumbed to acetaminophen intoxication. Shaded areas indicate 95% compatibility intervals for the survival estimates (lines). Differences between the 5th and 95th percentiles of dosage (B) and body condition (C) demonstrate the size of the respective effect.

observed thrashing until they flipped over becoming limp with their mouth open and occasionally taking prolonged gasps of air. Males often had their hemipenes everted. Thrashing only occurred over a short period of time just prior to snake's death, while most of the time snakes showed no signs of stress.

Table 2. Comparison of studies evaluating oral toxicity of acetaminophen to reptiles. LD_{Lo} = lowest dose observed to be lethal to a study subject. LD_{Hi} = Dose above which all (LD₁₀₀) or most (LD₉₉ = 99%, LD₉₈=98%) subjects died.

Species and sample size*	Form	Minimum and Maximum Mass (g) and Dosage (mg/kg)	LD _{Lo} (mg/kg)	LD _{Hi} (mg/kg)	Source
California Kingsnake N=121	Powder in gelatin capsule	26.9–554 75.3–2230	94.2	LD ₁₀₀ =413 LD ₉₉ =133 LD ₉₈ =120	Current study
Brown Treesnake N=100	Uncoated tablet	29.1–320 70.8–8312	145	LD ₁₀₀ =1913 LD ₉₉ =1571 LD ₉₈ =593	Savarie P, USDA, unpublished data
Burmese Python N=21	Uncoated tablet	78.7–191 128–703	Not reported	LD ₁₀₀ =263	Mauldin and Savarie 2010
Nile Monitor N=24	Uncoated tablet (force-fed)	16.2–150 67.5–2438	Not reported	LD ₁₀₀ =522	Mauldin and Savarie 2010
Black Spiny-tailed Iguana N=36	Uncoated tablets	Not specified 40–240 mg	3/8 mort $\bar{x}$ =709 (SE=201)	Not determined	Avery et al. 2011
Jackson's Chameleon N=35	Crushed tablets in snail shells	Not specified 200–2000	800 (2/6 mort)	LD ₁₀₀ †=2000 (8/8 mort)	Van Kleeck and Holland 2018

* Only dosed animals are counted. † This study sought to find the dosage above which 95% or more of subjects would die within 48 hours (euthanized after 48 hours); all 8 individuals receiving this dosage died.

Discussion

Our results demonstrate that acetaminophen is a highly effective oral toxicant for California kingsnakes invasive to Gran Canaria, and that the 60-mg dose was sufficient to lethally intoxicate all 40 snakes in the treatment group. Table 2 summarizes how our methods and results compare to those of previous evaluations of acetaminophen toxicity to reptiles. In addition to methemoglobinemia brought about by liver damage, Mathies and Mauldin (2020) also found several serum enzymatic indicators of liver damage and indication of possible muscle damage in brown treesnakes treated with acetaminophen. Liver damage was also noted in chameleons lethally dosed with acetaminophen (Van Kleeck and Holland 2018). Extreme sensitivity of snakes to acetaminophen is based on a lack of genes coding for enzymes that are responsible for biotransformation of acetaminophen in other vertebrates; other reptiles also share this trait to a lesser extent, and all reptiles appear to have lower tolerance for acetaminophen than most mammals (Van den Hurk and Kerckamp 2019).

The heaviest snake in our study was 554 g; to reach the 133 mg/kg LD₉₉, the snake would require a dose of approximately 74 mg. This snake was in our 80-mg treatment group and died, as did the next-heaviest snake (542 g) in the 60-mg group with a dosage of 111 mg/kg. The reported range of kingsnake masses reaches up to 2,268 g (Bartz 2012); at this mass, a kingsnake might be expected to need over 300 mg to be 99% confident of mortality. Within our sample, the heaviest snakes tended to be male (8 of 10 greater than 400 g). Fisher et al. (2019) found that adult California kingsnakes on Gran Canaria had greater body mass than those in their native range, with up to 23% greater average mass within the top decile compared to snakes caught in southern California. In their sample of 519

adult snakes from Gran Canaria, the heaviest was 770 g; an 80-mg tablet would equal a dosage of 104 mg/kg, and a dose of 103 mg would be required to put a snake of that size over the LD₉₉ of 133 mg/kg derived in this study. Fortunately, in experiments where a small number of larger brown treesnakes had survived ingestion of a dead newborn mouse (DNM) treated with 80 mg of acetaminophen, the survivors readily consumed another treated DNM a week later and all died (Nafus and Siers 2017). It is not yet known whether death after a second dose is a function of sampling variance (e.g., chance of surviving a 95% probability of death twice is only 0.25%) or whether there are lingering effects of the first dose that make snakes more susceptible to a subsequent dose.

Regurgitation of dosed bait mice does not increase probability of surviving. Based on the unreported data summarized in Table 2 (Savarie P, USDA, *unpublished data*), 26/104 (25%) of dosed brown treesnakes regurgitated (all of which died), compared to 83.6% of California kingsnakes in our study. This difference could be the result of some innate physiological differences between the two species; however, the USDA data also indicated that differences in tablet coating affected regurgitation rates. After controlling for survival and dosage (neither of which influenced regurgitation rates), snakes dosed with tablets that had an enteric coating regurgitated more frequently than those receiving uncoated tablets or tablets coated with beeswax ($p = 0.001$ and 0.039 , respectively; logistic regression with Tukey's HSD test). It is possible that our higher rate of regurgitation is associated with the powdered form of acetaminophen in gel caps we used in this study. This difference appears not to influence efficacy, since both studies exhibited very high mortality rates. Our control treatment did not incorporate an inert powder into the placebo gelatin capsules; it is possible that regurgitation may have been caused by a release of powder into the digestive system irrespective of presence of the active ingredient. In practice, a high regurgitation rate may increase environmental risks if regurgitated baits still contain high concentrations of acetaminophen and are subsequently ingested by nontarget species.

Although a 60-mg dose was 100% lethal in our test group, the heaviest snake we tested at that dose was 542 g. While it is desirable to minimize toxic inputs into the environment, in order to ensure efficacy for rare larger snakes it may be prudent to consider the 80-mg uncoated acetaminophen tablets currently prescribed for brown treesnake control. A practical advantage of this approach would be that risk analyses and other test results amassed in support of the USEPA registration of acetaminophen for brown treesnake control might be accepted as part of a registration process for acetaminophen for control of other invasive snakes, including California kingsnakes in the Canary Islands.

The use of any product as a pesticide is likely to be strongly regulated by jurisdictional law. Our results should in no way encourage the use of any

drug or pesticide in a manner inconsistent with the product label. Any California kingsnake or other invasive reptile control program must comply with all environmental and other applicable regulations of the appropriate jurisdictions, and any suspected misuse of a product for pesticidal applications should be reported to the appropriate authorities.

Managers confronting the brown treesnake invasion of Guam did not have an effective oral toxicant until well after the snake had invaded all habitats across Guam's landscapes and the greatest damages had already been caused. In that sense, managers trying to stem the California kingsnake invasion of the Canary Islands have an advantage. However, while an integral tool in interdiction and control (Clark et al. 2018), and growing in utility as new application methods are devised (e.g., Siers et al. 2019), acetaminophen has not been a panacea for brown treesnake management in Guam. Nor do we suggest that it may be the solution for control of California kingsnakes in the Canary Islands. Nontarget risks are very limited on Guam (Johnston et al. 2002), while the fauna in the Canaries is more diverse and potentially susceptible to nontarget poisoning, such as buzzards (*Buteo buteo* Linnaeus, 1758), ravens (*Corvus corax* Linnaeus, 1758), invasive rodent species, and feral cats and dogs. Canary Islands have high rates of endemism (Emerson 2003), which may require increased caution when considering acetaminophen field use (especially if California kingsnakes have an increased regurgitation response to acetaminophen). Lizards on Gran Canaria are either frugivores or insectivores, so potentially the concern may be limited to Gran Canaria avian species.

With all invasive species concerns, there is no substitute for prevention. Prudent management of the California kingsnake invasion in the Canaries should begin with ensuring that the "contagion" is not spread to other islands and habitats. LIFE+ Lampropeltis's objectives were to research California kingsnake behavior, implement a capture program, inform the public, and advocate for increased regulation on animal imports. All the objectives were met, including influencing legislation to prohibit live or dead colubrid snake possession in the Canary Islands, Ibiza, and Formentera (Cabrera-Pérez et al. 2012), but meeting these objectives did not accomplish their main objective of eradicating or even reducing California kingsnake populations. Contrary to LIFE+ Lampropeltis's main objective, the most recent established population, Maspalomas, occurred during LIFE+ Lampropeltis's leadership and California kingsnake's populations continue to expand with snakes being found in new areas daily (LIFE+ Lampropeltis 2012). New priorities should be focused on port biosecurity to prevent California kingsnakes from island-hopping to other Canary Islands as has occurred on Guam for prevention of further spread of Brown Treesnakes (Clark et al. 2018). There are currently no plans, to our knowledge, to increase port biosecurity on Gran Canaria.

As with brown treesnake interdiction, acetaminophen baits may be a cost-effective tool to prevent spread off the island. However, unwarranted enthusiasm for acetaminophen as a control tool should not lend a false sense of confidence in the ability to protect native species at risk from California kingsnake predation (e.g., the Gran Canaria giant lizard) and proactive conservation measures should be taken to prevent extirpation. If nontarget risks are duly evaluated and mitigated, and if mitigation doesn't dilute more direct interdiction and conservation efforts, acetaminophen baits may be useful in reducing or eliminating California kingsnakes and the damage they cause in the Canary Islands.

Acknowledgements

We would like to thank Calvin Schaefer, Ana Chapman, and Caleb Stevens for aiding in feeding snakes during trials, Océane Da Cunha and Dawei Han for their assistance transporting snakes to Truman State University, and the three anonymous reviewers during the revision of our manuscript.

Funding Declaration

The authors received no specific funding for this work.

Authors' Contribution

JF and CEM designed the experiment and methodology; JF collected data; JF and SRS analyzed and interpreted data.

References

- Amrhein V, Greenland S, McShane B (2019) Retire statistical significance. *Nature* 567: 305–307, <https://doi.org/10.1038/d41586-019-00857-9>
- Anderson DR (2008) Model based inference in the life sciences: a primer on evidence. Springer Science & Business Media LLC, New York, USA, 184 pp, <https://doi.org/10.1007/978-0-387-74075-1>
- Arnold TW (2010) Uninformative parameters and model selection using Akaike's Information Criterion. *Journal of Wildlife Management* 74: 1175–1178, <https://doi.org/10.2193/2009-367>
- Avery ML, Eisemann JD, Keacher KL, Savarie PJ (2011) Acetaminophen and zinc phosphide for lethal management of invasive lizards *Ctenosaura similis*. *Current Zoology* 57: 625–629, <https://doi.org/10.1093/czoolo/57.5.625>
- AVMA (2013) AVMA guidelines for the euthanasia of animals: 2013 Edition. American Veterinary Medical Association, Schaumburg, Illinois, USA, 102 pp
- Bartz S (2012) *Lampropeltis getula*. Animal Diversity Web. <https://animaldiversity.org> (accessed 26 June 2019)
- Bonnet X, Naulleau G (1995) Estimation of body reserves in living snakes using a Body Condition Index (BCI). In: Llorente GA, Montori A, Santos X, Carretero MA (eds), *Scientia Herpetologica*. Barcelona, pp 237–240
- Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer-Verlag, New York, USA, 488 pp
- Bushar LM, Reynolds RG, Tucker S, Pace LC, Lutterschmidt WI, Odum RA, Reinert HK (2015) Genetic characterization of an invasive *Boa constrictor* population on the Caribbean island of Aruba. *Journal of Herpetology* 49: 602–610, <https://doi.org/10.1670/14-059>
- Cabrera-Pérez MÁ, Gallo-Barneto R, Esteve I, Patiño-Martínez C, López-Jurado LF (2012) The management and control of the California kingsnake in Gran Canaria (Canary Islands): Project LIFE+ Lampropeltis. *Aliens: The Invasive Species Bulletin* 32: 20–28
- Campbell EW, Yackel Adams AA, Converse SJ, Fritts TH, Rodda GH (2012) Do predators control prey species abundance? An experimental test with brown treesnakes on Guam. *Ecology* 93: 1194–1203, <https://doi.org/10.1890/11-1359.1>
- Clark L, Savarie PJ (2012) Efficacy of aerial broadcast baiting in reducing brown tree snake numbers. *Human-Wildlife Interactions* 6: 212–221, <https://doi.org/10.26077/m7n9-pz85>

- Clark L, Savarie PJ, Shivik JA, Breck SW, Dorr BS (2012) Efficacy, effort, and cost comparisons of trapping and acetaminophen-baiting for control of brown tree snakes on Guam. *Human-Wildlife Interactions* 6: 222–236
- Clark L, Clark CS, Siers S (2018) Brown Tree Snakes Methods and Approaches for Control. In: Pitt WC, Beasley J, Witmer GW (eds), *Ecology and Management of Terrestrial Vertebrate Invasive Species in the United States*. CRC Press, LLC, Boca Raton, USA, pp 107–134, <https://doi.org/10.1201/9781315157078-7>
- Dorcas ME, Wilson JD, Reed NR, Snow RW, Rochford MR, Miller AM, Meshaka WE Jr, Andreadis PT, Mazzotti FJ, Romagosa CM, Hart KM (2012) Severe mammal declines coincide with proliferation of invasive Burmese pythons in Everglades National Park. *Proceedings of the National Academy Science* 109: 2418–2422, <https://doi.org/10.1073/pnas.1115226109>
- Dorr BS, Clark CS, Savarie PJ (2016) Aerial application of acetaminophen-treated baits for control of brown tree snakes (RC-200925; NWRC Study Number: QA-1828). Final Report, US-Department of Defense ESTCP, 62 pp
- Emerson BC (2003) Genes, geology and biodiversity: faunal and floral diversity on the island of Gran Canaria. *Animal Biodiversity and Conservation* 26(1): 9–20
- Filz KJ, Bohr A, Lötters S (2018) Abandoned Foreigners: is the stage set for exotic pet reptiles to invade Central Europe? *Biodiversity and Conservation* 27: 417–435, <https://doi.org/10.1007/s10531-017-1444-3>
- Fisher SR, Fisher RN, Alcaraz SE, Gallo-Barneto R, Patino-Martinez C, Jurado LL, Rochester CJ (2019) Life-history comparisons between the native range and an invasive island population of a colubrid snake. In: Veitch CR, Clout MN, Martin AR, Russell JC, West CJ (eds), *Island invasives: scaling up to meet the challenge*, International Union for Conservation of Nature, Gland, Switzerland, pp 326–331
- Fritts TH (1993) The common wolf snake, *Lycodon aulicus capucinus*, a recent colonists of Christmas Island in the Indian Ocean. *Wildlife Research* 20: 261–265, <https://doi.org/10.1071/WR9930261>
- Johnston JJ, Savarie PJ, Primus TM, Eisemann JD, Hurley JC, Kohler DJ (2002) Risk assessment of an acetaminophen baiting program for chemical control of brown tree snakes on Guam: Evaluation of baits, snake residues, and potential primary and secondary hazards. *Environmental Science & Technology* 36: 3827–3833, <https://doi.org/10.1021/es015873n>
- Kraus F (2009) Global trends in alien reptiles and amphibians. *Aliens: The Invasive Species Bulletin* 28: 13–18
- LIFE+ Lampropeltis (2012) Control de la especie invasora la culebra real de California (*Lampropeltis getula californiae*) en la isla de Gran Canaria. <https://www.lifelampropeltis.com> (accessed 5 August 2017)
- Markel RG (1989): *Kingsnakes and Milk Snakes*. T.F.H. Publications, Neptune City, New Jersey, 94 pp
- Mathies T, Mauldin RE (2020) Lethal methaemoglobinaemia in the invasive brown treesnake after acetaminophen ingestion. *Scientific Reports* (in press)
- Mauldin RE, Savarie PJ (2010) Acetaminophen as an oral toxicant for Nile monitor lizards (*Varanus niloticus*) and Burmese pythons (*Python molurus bivittatus*). *Wildlife Research* 37: 215–222, <https://doi.org/10.1071/WR08168>
- Monzón-Argüello C, Patiño-Martínez C, Christiansen F, Gallo-Barneto R, Cabrera-Pérez MÁ, Peña-Estévez MÁ, Lee PL (2015) Snakes on an island: independent introductions have different potentials for invasion. *Conservation Genetics* 16: 1225–1241, <https://doi.org/10.1007/s10592-015-0734-0>
- Nafus MG, Siers SR (2017) Efficacy and weatherability of externally-affixed acetaminophen tablets on Brown Treesnake baits. Unpublished Final Report QA-2768. USDA, APHIS, WS, NWRC. Hilo, Hawaii, USA, 7 pp + appendix
- Perry G, Pierce J, Griffin D, van Buurt G, Lazell J (2003) Geographic distribution: *Elaphe guttata guttata*. *Herpetological Review* 34: 264
- Pether J, Mateo JA (2007) La culebra real (*Lampropeltis getulus*) en Gran Canaria, otro caso preocupante de reptil introducido en el Archipiélago Canario. *Boletín de la Asociación Herpetológica Española* 18: 20–23
- Petren K, Case TJ (1996) An experimental demonstration of exploitation competition in an ongoing invasion. *Ecology* 77: 118–132, <https://doi.org/10.2307/2265661>
- Pinya S, Carretero MA (2011) The Balearic herpetofauna: a species update and a review on the evidence. *Acta Herpetologica* 6: 59–80, https://doi.org/10.13128/Acta_Herpetol-9579
- R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org>
- Reed RN, Tucker AD (2012) Determining age, sex, and reproductive condition. In: Foster M, McDiarmid R (eds), *Reptile biodiversity: Standard methods for inventory and monitoring*. University of California Press, Berkeley & Los Angeles, California, USA, pp 151–163
- Rodda GH, Fritts TH (1992) The impact of the introduction of the colubrid snake *Boiga irregularis* on Guam's lizards. *Journal of Herpetology* 26: 166–174, <https://doi.org/10.2307/1564858>

- Rodda GH, Savidge JA (2007) Biology and impacts of Pacific Island invasive species. 2. *Boiga irregularis*, the brown tree snake (Reptilia: Colubridae). *Pacific Science* 61: 307–324, [https://doi.org/10.2984/1534-6188\(2007\)61\[307:BAIOPI\]2.0.CO;2](https://doi.org/10.2984/1534-6188(2007)61[307:BAIOPI]2.0.CO;2)
- Rodda GH, Fritts TH, Conry PJ (1992) Origin and population growth of the brown tree snake, *Boiga irregularis*, on Guam. *Pacific Science* 46: 46–57
- Rodda GH, Fritts TH, Chiszar D (1997) The disappearance of Guam's wildlife. *BioScience* 47: 565–574, <https://doi.org/10.2307/1313163>
- Rogers H, Lambers JHR, Miller R, Tewksbury JJ (2012) 'Natural experiment' demonstrates top-down control of spiders by birds on a landscape level. *PLoS ONE* 7, <https://doi.org/10.1371/journal.pone.0043446>
- Rogers HS, Buhle ER, HilleRisLambers J, Fricke EC, Miller RH, Tewksbury JJ (2017) Effects of an invasive predator cascade to plants via mutualism disruption. *Nature Communications* 8: 14557, <https://doi.org/10.1038/ncomms14557>
- Ruell EW, Niebuhr CN, Sugihara RT, Siers SR (2019) An evaluation of the registration and use prospects for four candidate toxicants for controlling invasive mongooses (*Herpestes javanicus auropunctatus*). *Management of Biological Invasions* 10: 573–596, <https://doi.org/10.3391/mbi.2019.10.3.11>
- Savarie PJ, Clark L (2006) Evaluation of bait matrices and chemical lure attractants for brown tree snakes. *Proceedings of the Vertebrate Pest Conference* 22: 483–488, <https://doi.org/10.5070/V422110077>
- Savarie PJ, York DL, Hurley JC, Volz S, Brooks JE (2000) Testing the dermal and oral toxicity of selected chemicals to brown tree snakes. *Proceedings of the Vertebrate Pest Conference* 19: 139–145, <https://doi.org/10.5070/V419110219>
- Savarie PJ, Shivik JA, White GC, Hurley JC, Clark L (2001) Use of acetaminophen for large-scale control of brown tree snakes. *Journal Wildlife Management* 65: 356–365, <https://doi.org/10.2307/3802916>
- Savidge JA (1987) Extinction of an island forest avifauna by an introduced snake. *Ecology* 68: 660–668, <https://doi.org/10.2307/1938471>
- Siers SR, Savidge JA, Reed RN (2017) Quantile regression of microgeographic variation in population characteristics of an invasive vertebrate predator. *PLoS ONE* 12: e0177671, <https://doi.org/10.1371/journal.pone.0177671>
- Siers SR, Pitt WC, Eisemann JD, Clark L, Shiels AB, Clark CS, Gosnell RJ, Messaros MC (2019) In situ evaluation of an automated aerial bait delivery system for landscape-scale control of invasive brown treesnakes on Guam. In: Veitch CR, Clout MN, Martin AR, Russell JC, West CJ (eds), *Island invasives: scaling up to meet the challenge*. IUCN Occasional Paper SSC no. 62. Gland, Switzerland, pp 348–355
- Silva-Rocha I, Salvi D, Sillero N, Mateo JA, Carretero MA (2015) Snakes on the Balearic Islands: An Invasion Tale with Implications for Native Biodiversity Conservation. *PLoS ONE* 10: e0121026, <https://doi.org/10.1371/journal.pone.0121026>
- Snow RW, Krysko KL, Enge KM, Oberhofer L, Warren-Bradley A, Wilkins L (2007) Introduced populations of *Boa constrictor* (Boidae) and *Python molurus bivittatus* (Pythonidae) in southern Florida. In: Henderson RW, Powell R (eds), *Biology of Boas and Pythons*. Eagle Mountain Publishing, Eagle Mountain, Utah, USA, pp 365–386
- Stebbins RC (1998) Peterson Field Guide to Western Reptiles and Amphibians. Houghton Mifflin Company, Boston, USA, 560 pp
- Stuart YE, Campbell TS, Hohenlohe PA, Reynolds RG, Revell LJ, Losos JB (2014) Rapid Evolution of a Native Species Following Invasion by a Congener. *Science* 346: 463–466, <https://doi.org/10.1126/science.1257008>
- Tonge S (1990) The past, present and future of the herpetofauna of Mauritius. *Bulletin of the Chicago Herpetological Society* 25: 220–226
- Van den Hurk P, Kerkkamp HM (2019) Phylogenetic origins for severe acetaminophen toxicity in snake species compared to other vertebrate taxa. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 215: 18–24, <https://doi.org/10.1016/j.cbpc.2018.09.003>
- Van Kleeck M, Holland BS (2018) Chemical control of the invasive Jackson's chameleon (*Trioceros jacksonii xantholophus*) in Hawaii. *International Journal of Pest Management* 64: 236–242, <https://doi.org/10.1080/09670874.2017.1386334>
- Valido A, Nogales M, Medina FM (2003) Fleshy fruits in the diet of Canarian lizards *Gallotia galloti* (Lacertidae) in a xeric habitat of the island of Tenerife. *Journal of Herpetology* 37: 741–748, <https://doi.org/10.1670/15-02AN>
- Wiles GJ, Bart J, Beck RE, Aguon CF (2003) Impacts of the brown tree snake: Patterns of decline and species persistence in Guam's avifauna. *Conservation Biology* 17: 1350–1360, <https://doi.org/10.1046/j.1523-1739.2003.01526.x>