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# Testing toxicants and baits to control small invasive lizards

Fred Kraus[1,*], Robert Sugihara[2] and Shane Siers[3]

[1]*Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA*
[2]*USDA APHIS Wildlife Services, National Wildlife Research Center, 210 Amau'ulu Rd., Hilo, HI, USA*
[3]*USDA APHIS Wildlife Services, National Wildlife Research Center, c/o Wildlife Services Guam State Office, 233 Pangelinan Way, Barrigada, GU, USA*

Author e-mails: *fkraus@umich.edu (FK), robert.t.sugihara@usda.gov (RS), shane.r.siers@usda.gov (SS)*

**Corresponding author*

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## Abstract

Several species of small invasive lizards form high densities in their invaded ranges and threaten native biodiversity in a variety of ways. Little attention has, however, been devoted toward identifying tools to control populations of these lizards for conservation purposes. Some of these invasive lizards or their close relatives consume a variety of sweet food items, either natural or human-made. Thus, we conducted experimental laboratory trials to identify the potential for sweetened solutions, pastes, or fruit products to serve as attractive baits for two invasive lizard species, and we evaluated the potential effectiveness of acetaminophen and alpha-chloralose to serve as toxicants when mixed in such baits. Using paired-choice tests, we identified red baits (as corn syrups, sweetened pastes, or strawberries) as the most effective baits for *Anolis carolinensis* and white corn syrup as the most effective bait for *Hemidactylus frenatus*. Bait attractiveness was improved by the addition of vanilla flavoring. We assessed the LD100 of acetaminophen and alpha-chloralose to *A. carolinensis*, *H. frenatus*, and *Lampropholis delicata* by oral application of solutions of known concentration. Acetaminophen proved infeasible for use as a toxicant in sweet baits because of its low aqueous solubility and high concentrations needed to attain lethality; alpha-chloralose was more successful in this regard because of its greater toxicity and smaller quantities needed for use. However, keeping the toxin in suspension remains a problem. Trials combining alpha-chloralose with some of the more effective baits provided some degree of lethality among samples but indicated that further work is required in order to identify the best bait matrix for control projects in a field setting. We recommend next steps to advance development of an effective toxicant+bait system for controlling small invasive lizards that build off our results.

**Key words:** acetaminophen, alpha-chloralose, *Anolis carolinensis*, *Hemidactylus frenatus*, *Lampropholis delicata*

## Introduction

Several genera of small invasive lizards (e.g., *Anolis*, *Carlia*, *Hemidactylus*) can be disruptive to native ecosystems because of their high densities and biomass (Kraus 2009, 2015). For example, alien *Anolis carolinensis* can reach densities up to 2570 lizards/ha in the Ogasawara Islands (Okochi et al. 2006), and *Anolis sagrei* can reach 12,000 lizards/ha and 43 kg/ha in Florida (Campbell and Echternacht 2003). Ecological impacts from such

lizards include endangerment of native species resulting from predation (e.g., *Anolis*, Karube 2004a, b, 2005; Karube and Suda 2004; Makihara et al. 2004; Takakuwa and Suda 2004; Yoshimura and Okochi 2005; Okochi et al. 2006), competition (e.g., *Hemidactylus*, Cole et al. 2005), hybridization (e.g., *Podarcis*, *Plestiodon*, Capula 1993; Capula et al. 2002; Okamoto et al. 2013; D'Amico et al. 2018), or trophic subsidization of other invasive species that themselves threaten native wildlife (e.g., *Carlia* and various geckos subsidizing brown treesnakes, *Boiga irregularis*, Fritts and Rodda 1998; McCoid 1999; Rodda et al. 1999a, b). These threats are especially pronounced on oceanic islands. Given the large number of small lizard species that have been introduced around the world (> 200, Kraus 2009; FK *unpubl. data*), it is likely that other populations of these and similar species are having negative ecological impacts, but they remain as yet unrecognized and unstudied.

Beyond effects on native fauna, some geckos, such as several *Hemidactylus* species, are commensal with humans, inhabiting their houses and defecating inside. This behavior raises health concerns for transmission of human *Salmonella* infection by contamination of food and water exposed to gecko feces (Chan et al. 1982; Mermin et al. 2004). Further, even though they are not human commensals, large populations of *Carlia* and *Anolis* lizards on Guam are thought to contribute to high prevalence of virulent strains of human salmonellosis there because of their prevalence in yards where humans play or relax (Haddock et al. 1990; Haddock and Nocon 1993). And *A. carolinensis* maintains high *Salmonella* infection rates on Chichijima, Ogasawara Islands, raising concerns that fecal contamination in and around houses could spread the disease to humans there (Sumiyama et al. 2014).

These negative impacts make control of dense populations of small alien lizards desirable in at least some circumstances, but attempts to identify effective control techniques are rare. Similarly, tools to stop further accidental introductions of such lizards via cargo transportation into currently uninvaded areas would be beneficial. In contrast to larger invasive squamates such as iguanas, brown treesnakes, or pythons, no attempt has yet been made to identify control methods for small lizards other than labor-intensive hand-capture or use of sticky traps (e.g., Toda et al. 2010). However, the high densities that most of these species can form make these traditional capture methods infeasible for population removal. A control method less labor intensive would, therefore, be desirable. A toxin-laced bait could theoretically provide such an alternative, given their history of success controlling a diversity of mammals (cf. Veitch and Clout 2002; Veitch et al. 2011, 2019) and birds (Linz et al. 2015, 2018; Avery and Lockwood 2018) and their development for local control of some reptiles (Siers et al. 2019). Although most small lizards are insectivorous, a number of the most invasive lizards (e.g., *Anolis*, several gecko genera) is also attracted to fruits, nectar, and other sweet substances (Olesen and Valido 2003; Hansen et al. 2006) and could conceivably be baited with such items. Toxicant work on small

lizards is unreported in the literature yet offers potential for large-scale and effective control if a safe and effective toxicant and delivery system could be found. Both liquid and paste delivery systems (baits) require testing because each is likely to be most effective in different environments: liquids may be more readily deployable in feeding stations in the field, and pastes more usable in cargo containers and around buildings.

Acetaminophen is toxic to several squamate species (Savarie et al. 2000, 2001; Mauldin and Savarie 2010) yet relatively safe to mammals; it has only once been tested for efficacy against small-sized lizards in an LD50 study (Wairepo 2015). Three lines of evidence suggest that alpha-chloralose may also be an effective toxicant for small lizards: (1) it is the active constituent of a commercial, proprietary product in La Réunion and Mauritius marketed for killing commensal geckos in houses, although no published data are available on its efficacy; (2) a few lizards have been found dead subsequent to alpha-chloralose control operations against birds in New Zealand (T. Whitaker 2008, *pers. comm.*; J. Reardon 2019, *pers. comm.*); and (3) direct application of alpha-chloralose to the mouths of *Hemidactylus* geckos on Norfolk Island resulted in death (T. Whitaker 2008, *pers. comm.*). Despite these suggestive anecdotes, no published scientific data are available for the effects of alpha-chloralose on lizards.

The objectives of this study were threefold. First, to identify acetaminophen and alpha-chloralose doses sufficient for 100% mortality of test specimens (LD100's) of small invasive lizards of the genera *Anolis*, *Hemidactylus*, and *Lampropholis*. Second, to identify baits effective in attracting these lizards to ingest each substance. Third, to determine whether the combination of an effective toxin mixed with an attractive bait could prove successful in killing lizards.

## Materials and methods

In July–August 2018 and July 2019, we hand-collected *Anolis carolinensis*, *Hemidactylus frenatus*, and *Lampropholis delicata* at urban and rural locations in or near Hilo and Volcano, Hawaii Island, returned them to the National Wildlife Research Center Field Station in Hilo, maintained them briefly in 7.6-liter plastic terraria (305 × 194 × 205 mm, Carolina Biological Supply), and used them in experiments to identify effective baits and dosages of acetaminophen and alpha-chloralose. We maintained the lizard room at 23–27 °C with a 12 hr on/12 hr off light cycle (0600–1800 h light). We did not record room humidity, but it typically ranges from 85–100% (RS, *pers. obs.*). We provided water ad libitum but no supplemental food. To minimize numbers of lizards needed for these trials, we used each *Anolis* and *Hemidactylus* lizard 2–3 times: first in one or two of the **feeding trials** (testing different non-toxic formulations [liquid, paste] if used in two trials so as to obviate obtaining a learned response), and finally in the LD100 **toxicity trials**. We obtained a new set of animals for the **toxic bait trials**.

We weighed lizards with a Mettler PE 3600 to the nearest 0.01 g before and after their trial period. We acclimated lizards to their cages overnight before being assigned to tests arbitrarily in the order in which they were retrieved from collection bags and housed.

The target lizards or their close relatives are known to consume fruits, nectar, sap, or sugared human products (McCoid and Hensley 1993; Poulin et al. 1995; Henkel and Schmidt 2000; Cooper and Vitt 2002; McCoy 2006; Valido 2006; Valido and Olesen 2019), so sugared products provide a reasonable system to test as a potential bait for toxin delivery. However, the solubility of both acetaminophen and alpha-chloralose in water is low (acetaminophen 1–5 mg/ml at 22 °C, alpha-chloralose 4 mg/ml at 15 °C), and, at the dosages known or suspected for these products to be toxic to squamates (0.2–2.6 μg/g), neither compound will dissolve completely, so we investigated baits that could potentially hold each in suspension. For this purpose we relied on corn syrup and sweetened pastes.

*Feeding trials.* We designed tests to examine (1) solution/paste color most attractive to lizards, (2) whether bait attractiveness could be improved by addition of a scent, (3) whether an inert protein bait, fruit pulp, or honey were more effective in attracting lizards than were the sweet baits, and (4) whether either liquid or paste baits were more effective or equally effective as a delivery system. We made solutions by adding commercial food colorings and/or scents to 100% corn syrup (Western Family light). In the first year we made pastes by adding food coloring and/or scents to a paste made by mixing Aerosil 200 fumed silica (Sigma) with enough mineral oil to produce the desired consistency. In the second year we substituted corn syrup for the mineral oil to improve attractiveness of the paste. Additionally, we also evaluated the attractiveness of strawberry jelly (Western Family), guava jelly (Hawaiian Sun), mashed papaya, mashed strawberries, honey, and tinned cat food (Little Friskies); we chose these tests based on either anecdotal reports of certain lizards feeding on these items or their similarity to items known to be taken. We devised stepwise paired trials for each species, testing alternate colors, scents, and formulations (e.g., corn syrup vs. jelly or jam or cat food); we conducted paste trials varying color and scents separately. For most of the resulting 28 trials, we used ten animals of mixed sex (Tables 1–3, Supplementary material Tables S1–S3).

In the first year's trials, we dispensed corn-syrup (CS) solutions and pastes in 6 ml plastic petri dishes in which access to the liquid/paste was provided by drilling a 12.6 mm hole in the lid; in the second year, we dispensed these materials in shortened polypropylene collection tubes of *ca*. 23–24 mm height and 14 mm inner diameter (16.5 mm OD) placed in holes drilled in small 2.5 × 10 cm wooden blocks. We made this change to determine whether consumption differences could be more sensitively measured if less volume were used. These diameter openings sufficiently accommodated the heads of all lizards tested. We dispensed test substances

**Table 1.** Summary of feeding trials for *Anolis carolinensis*. Samples (N) were of roughly equal sex ratios. Mean and SD are for individual consumption values after controlling for evaporation (difference from evaporative controls). P-values are based on a paired sampled two-sided *t*-test, with values ≤ 0.10 reported. Significantly preferred substances are highlighted in bold. Straw = strawberry essence.

| Trial | N | Mean | SD | Mean Difference | *P*-value |
|---|---|---|---|---|---|
| Red corn syrup | 10 | −0.010 | 0.0279 | 0.021 | NS |
| *vs.* orange corn syrup | | −0.031 | 0.1456 | | |
| Strawberry jam | 10 | 0.024 | 0.0470 | −0.007 | NS |
| *vs.* red corn syrup | | 0.031 | 0.0955 | | |
| Guava jelly | 10 | −0.021 | 0.0110 | −0.086 | 0.006 |
| ***vs.* orange corn syrup** | | 0.065 | 0.0826 | | |
| **Red paste in mineral oil** | 7 | 0.014 | 0.0288 | 0.030 | 0.038 |
| *vs.* orange paste in mineral oil | | −0.016 | 0.0079 | | |
| **Red corn syrup + Vanilla** | 10 | 0.048 | 0.0529 | 0.039 | 0.049 |
| *vs.* clear corn syrup | | 0.009 | 0.0264 | | |
| Cat food | 10 | −0.011 | 0.0331 | −0.041 | NS |
| *vs.* red corn syrup | | 0.03 | 0.0869 | | |
| Red paste in corn syrup | 10 | 0.016 | 0.0177 | 0.0149 | 0.052 |
| *vs.* yellow paste in corn syrup | | 0.001 | 0.0081 | | |
| Orange paste in corn syrup | 10 | −0.002 | 0.0044 | −0.002 | 0.022 |
| *vs.* **yellow paste in corn syrup** | | 0.000 | 0.0038 | | |
| Red paste in corn syrup | 10 | −0.000 | 0.0042 | 0.003 | 0.091 |
| *vs.* red paste in corn syrup + Vanilla | | −0.003 | 0.0035 | | |
| Red corn syrup | 8 | 0.121 | 0.1932 | 0.100 | NS |
| *vs.* red corn syrup + Straw | | 0.021 | 0.0640 | | |
| **Red paste in corn syrup** | 10 | 0.092 | 0.2667 | 0.020 | < 0.001 |
| *vs.* red paste in corn syrup + Straw | | 0.072 | 0.2682 | | |
| Clear corn syrup | 9 | −0.033 | 0.1462 | −0.067 | NS |
| *vs.* honey | | 0.034 | 0.0922 | | |
| Mashed papaya | 10 | −0.017 | 0.0229 | −0.062 | 0.021 |
| *vs.* **mashed strawberry** | | 0.045 | 0.0715 | | |

as paired choices with similar test-substance controls placed in an empty terrarium to correct consumption values for moisture loss or gain during each trial. Color trials compared red, orange, yellow, and white solutions and pastes. We assessed scents by adding them to that color of solution/paste previously identified as most attractive. We tested artificial vanilla (Value Corner) and strawberry scent (Generessence) obtained from International Flavors & Fragrances, with 10 µl of the test scent added to each petri dish of solution, or an equivalent amount added at an identical concentration to a larger batch of corn syrup or paste that was then aliquoted among trials. We held sugar concentration of solutions constant at 100% glucose (corn syrup) because of the need to suspend any potential toxicant in a dense support matrix. We held concentrations of sweeteners in pastes constant by adding 4g sucralose (Splenda) finely ground with a mortar and pestle to 20 g paste. We made colored solutions and pastes using commercial food colorings (McCormick brand) added to the preferred concentration of sugar solution, except that white coloration was produced by adding titanium dioxide—a non-toxic powder widely used in food products, toothpaste, cosmetics, and sunscreen solutions—at a concentration of 1 g $TiO_2$/200 ml corn syrup. We continued feeding trials for 48 h, and we measured the mass of liquid/paste placed with each lizard with a Mettler PE 3600 to the nearest 0.01 g at the beginning and end of each trial in the first year's trials

**Table 2.** Summary of feeding trials for *Hemidactylus frenatus*. Samples (N) were of roughly equal sex ratios. Mean and SD are for individual consumption values after controlling for evaporation (difference from evaporative controls). P-values are based on a paired sampled two-sided *t*-test, with values ≤ 0.10 reported. Significantly preferred substances are highlighted in bold.

| Trial | N | Mean | SD | Mean Difference | *P*-value |
|---|---|---|---|---|---|
| Red corn syrup | 10 | −0.001 | 0.0281 | 0.009 | NS |
| *vs.* orange corn syrup | | −0.010 | 0.0320 | | |
| Red corn syrup | 10 | 0.027 | 0.0368 | 0.012 | NS |
| *vs.* yellow corn syrup | | 0.015 | 0.0178 | | |
| Orange corn syrup | 10 | 0.051 | 0.0331 | 0.005 | NS |
| *vs.* yellow corn syrup | | 0.046 | 0.0241 | | |
| Red paste | 10 | 0.042 | 0.0473 | −0.016 | NS |
| *vs.* orange paste | | 0.058 | 0.0326 | | |
| Red paste | 10 | −0.001 | 0.0589 | −0.008 | NS |
| *vs.* yellow paste | | 0.007 | 0.0170 | | |
| Orange paste | 10 | −0.010 | 0.0211 | −0.018 | 0.014 |
| *vs.* **yellow paste** | | 0.008 | 0.0155 | | |
| Strawberry jam | 10 | −0.039 | 0.0269 | −0.107 | < 0.001 |
| *vs.* **red corn syrup** | | 0.068 | 0.0471 | | |
| Guava jelly | 9 | 0.008 | 0.0254 | −0.078 | 0.005 |
| *vs.* **red corn syrup** | | 0.087 | 0.0502 | | |
| White corn syrup | 10 | 0.027 | 0.0177 | 0.003 | NS |
| *vs.* red corn syrup | | 0.024 | 0.0341 | | |
| White corn syrup | 9 | 0.033 | 0.0316 | 0.021 | 0.088 |
| *vs.* clear corn syrup | | 0.012 | 0.0217 | | |
| **White corn syrup + vanilla** | 10 | 0.053 | 0.0254 | 0.051 | < 0.001 |
| *vs.* clear corn syrup | | 0.002 | 0.0301 | | |
| Cat food | 10 | −0.045 | 0.1007 | −0.082 | 0.024 |
| *vs.* **white corn syrup** | | 0.037 | 0.0365 | | |
| White corn syrup | 10 | −0.001 | 0.0138 | −0.013 | NS |
| *vs.* honey | | 0.011 | 0.0385 | | |

**Table 3.** Summary of feeding trials for *Lampropholis delicata*. Samples (N) were of roughly equal sex ratios. Mean and SD are for individual consumption values after controlling for evaporation (difference from evaporative controls). P-values are based on a paired sampled two-sided *t*-test, with values ≤ 0.10 reported.

| Trial | N | Mean | SD | Mean Difference | *P*-value |
|---|---|---|---|---|---|
| Mashed papaya | 10 | −0.086 | 0.4059 | −0.055 | NS |
| *vs.* mashed strawberry | | −0.031 | 0.0393 | | |

and with a Sartorius CP124S to the nearest 0.0001 g in the second year's trials. We subtracted the difference in mass from the control liquid/paste to give a measure of the amount of liquid/paste consumed by the lizard. Trials using the Sartorius in the second year frequently showed a weight gain over the course of the trial due to absorption of water from the air by the corn-syrup solutions and honey (in trials when used), which apparently wasn't detected by the lower-precision Mettler balance used in the first year. We corrected for these weight gains by subtracting weight gain in the trial vs. that in the control and using that difference as the measure of lizard consumption.

We recorded moisture-corrected consumption values for each bait for each individual. We tested differences in consumption between baits with a two-sided paired sample *t*-test, with each pair of observations being the consumption of each bait by an individual lizard. We also report sample estimates of the mean differences in consumption, along with means and standard deviations of consumption values for each treatment group. We used R Version 3.5.3 (R Core Team 2019) to perform statistical tests.

*Toxin trials.* We made stock solutions of acetaminophen (Spectrum Chemical, CAS 103-90-2) and alpha-chloralose (Sigma, 23120-100G-F) suspended in either an 80:20 (w/w) solution of corn syrup:water (for *Anolis* and *Hemidactylus*) or in water (for *Lampropholis*) in the first year or a 50:50 (w/w) solution of corn syrup:water for all species in the second year. These substrate differences were required by the difficulty of pipetting small quantities of the corn-syrup dilution with micro-pipettes. We found the 50% solutions to work best for this purpose for very small quantities. We agitated solutions with an electric mixer (Scientific Products, Cat. S8220) prior to delivery to improve suspension, and we delivered solutions to each lizard by gently opening its mouth (*Anolis* almost always gave a gape display of their own volition) and placing a quantity (6.2–276.8 µl, depending on size of animal) of the solution in the back of the mouth with a P100 or P1000 micro-pipette (Eppendorf). We used the larger pipette only for the largest lizards requiring > 100 µl of solution. We briefly held lizards with their mouths closed to ensure swallowing of the solution. In some instances, lizards would spit part of the solution out onto their lips; we then suctioned up that solution with the pipette and reinserted into the mouth. Tested lizards were an arbitrary mixed-sex subset of the animals used in the feeding trials, chosen as they were no longer needed for feeding trials.

We assessed the LD100 of each compound using a two-step method. First we obtained an initial estimate of toxicity using the up-and-down method (Dixon 1965; Bruce 1985). Once an initial lower lethal dosage was estimated, we verified its effectiveness using another ten animals so as to determine the importance of intra-specific variation in toxin resistance. If not all ten animals died, we then doubled the dose and tested another ten animals, which usually provided 100% toxicity for all tested animals. If that doubled dosage was close but not quite 100% effective, we then increased the dose slightly beyond that (true for alpha-chloralose for *H. frenatus*). Dosed animals were observed 2–4 times/day for two days to verify toxin effects. During each set of up-down tests we gave a single lizard an identical dosage of water (volume:weight) as a control. For the tests verifying dosage effectiveness that involved ten animals, we also gave ten controls water, though not necessarily on the same day due to limitations on the numbers of animals available at any given time. Dosages varied from 0.1–4.0 mg/g (Table 4), consistent with high-toxicity doses of acetaminophen known for other squamates (Savarie et al. 2000, 2001; Mauldin and Savarie 2010).

*Toxic bait trials.* We combined data from the first two experiments in preliminary tests to determine whether a toxin-laced bait solution, using some of the baits we found most effective in the feeding trials, could prove efficacious in killing *A. carolinensis* or *H. frenatus*. We individually offered lizards a free-choice trial of 1–4 different baits/ trial to see if they would consume sufficient bait to be killed (1–4 bait options for *A. carolinensis*, 1–2 options for *H. frenatus*) (Table 5). We measured trial success as the death

**Table 4.** Summary of toxin trials. In all cases, lizards given water controls survived 48 h (not shown). The best concentration for LD100 is shown in bold.

| Species | Compound | Concentration | N | Outcome |
|---|---|---|---|---|
| *Anolis carolinensis* | acetaminophen | 1 mg/g | 1 | survived 48 h |
| | | 2 mg/g | 1 | dead in 24 h |
| | | 1.8 mg/g | 1 | dead in 41.5 h |
| | | 2 mg/g | 1 | dead in 42 h |
| | | 1.9 mg/g | 1 | survived 48 h |
| | | 4 mg/g | 9 | 1 dead, 8 survived 48 h |
| | α-chloralose | 1 mg/g | 1 | dead in 24 h |
| | | 0.5 mg/g | 1 | dead in 24 h |
| | | 0.2 mg/g | 1 | dead in 24 h |
| | | 0.1 mg/g | 1 | initial knockdown, then recovered |
| | | 0.2 mg/g | 1 | initial knockdown, then recovered |
| | | 0.4 mg/g | 1 | survived 48 h |
| | | 1 mg/g | 10 | 3 dead, 7 survived 48 h |
| | | 4 mg/g | 10 | all dead within 18 h |
| | | **2 mg/g** | 10 | all dead within 18 h |
| *Hemidactylus frenatus* | acetaminophen | 2 mg/g | 1 | dead in 24 h |
| | | 1.5 mg/g | 1 | survived 42 h |
| | | 1.8 mg/g | 1 | dead in 23 h |
| | | 1.7 mg/g | 1 | survived 25 h |
| | | 1.8 mg/g | 10 | 1 dead, 9 survived 48 h |
| | | 3.6 mg/g | 8 | 1 dead, 7 survived 48 h |
| | α-chloralose | 0.2 mg/g | 1 | dead in 24 h |
| | | 0.15 mg/g | 1 | dead in 24 h |
| | | 0.1 mg/g | 1 | dead in 21.5 h |
| | | 0.05 mg/g | 1 | survived 48 h |
| | | 0.1 mg/g | 10 | 1 dead, 9 survived 48 h |
| | | 0.4 mg/g | 10 | 9 dead, 1 survived 48 h |
| | | **0.6 mg/g** | 10 | all dead within 45 h |
| *Lampropholis delicata* | acetaminophen | 2 mg/g | 1 | dead in18 h |
| | | 1.7 mg/g | 1 | survived 46 h |
| | | 1.8 mg/g | 1 | survived 45.5 h |
| | | 1.9 mg/g | 1 | survived 48 h |
| | | 4 mg/g | 6 | 2 dead, 4 survived 48 h |
| | α-chloralose | 0.1 mg/g | 1 | survived 42 h |
| | | 0.5 mg/g | 1 | dead in 21 h |
| | | 0.3 mg/g | 1 | dead in 22.5 h |
| | | 0.2 mg/g | 1 | dead in 18 h |
| | | 0.6 mg/g | 2 | dead in 19 h |
| | | **0.4 mg/g** | 10 | all dead within 24 h |

**Table 5.** Combined 5% alpha-chloralose + bait trials. In all cases, lizards given bait controls without toxin survived 45.5–47 h (not shown). Abbreviations as for Table 1.

| Species | Baits | N | Outcome |
|---|---|---|---|
| *Anolis carolinensis* | Red CS | 5 | all survived 96 h |
| | Red CS, red CS+V, honey, strawberry pulp | 5 | 1 dead in 42 h, remainder survived 96 h |
| | Red CS | 2 | all survived 48 h |
| | Red CS, red CS+V, honey, strawberry pulp | 3 | all survived 48 h |
| *Hemidactylus frenatus* | White CS+V | 10 | 2 dead in 45 h, 2 moribund but alive at 47 h, 6 survived 47 h |
| | White CS+V, honey | 10 | 1 toxified at 20 h but recovered by 45.5 h, 3 toxified but moving at 45.5 h, 6 survived 45.5 h |

of the tested lizards, but we also recorded lizards that showed initial signs of toxification (lethargy, immobility) but later recovered. As for the feeding trials, we weighed lizards, fasted them for one day, and then arbitrarily selected them for trials.

We euthanized all animals by inhalation of isoflurane (Isoflo, Zoetis Co.) placed on a cotton ball with the animal in a 1-liter jar at the end of their use and either froze them for further examination of parasites or fixed them in 10% formalin for deposition in the research collections at the University of Michigan Museum of Zoology (UMMZ 247209–67, 247277–330, 247338–68, 248459–574, 248577–86).

## Results

*Feeding trials.* Not all possible pairwise feeding trials could be conducted, but those conducted sequentially suggest that red coloration in concert with vanilla scent was the most effective bait for *Anolis carolinensis* (Tables 1, S1, S6), and white coloration in concert with vanilla scent was most effective for *Hemidactylus frenatus*, though honey was also attractive for that species (Tables 2, S2, S7). Time allowed for only one feeding trial with *Lampropholis delicata*, and that was non-significant (Tables 3, S3, S8). Consumption differences were small and often minimally different from losses in the control samples due to evaporation (Tables 1, 2). Trials with jellies, jams, and fresh or frozen fruits were generally less enticing, and trials with canned cat food did not show differences from evaporative weight loss in the control samples.

*Toxin trials.* Trials with acetaminophen were unsatisfactory and were terminated prior to identifying a reliable LD100. This is because high levels of acetaminophen were required for lizard toxicity (Tables 4, S4), but those concentrations could not be reliably placed into suspension for dosing the animals. Instead, the acetaminophen precipitated or flocculated and blocked the pipette tip. Trials with alpha-chloralose were more satisfactory, with dosages of 2.0 mg/g, 0.6 mg/g, and 0.4 mg/g all proving 100% lethal for *Anolis carolinensis*, *Hemidactylus frenatus*, and *Lampropholis delicata*, respectively (Tables 4, S4). In no case did animals treated with water controls die or show any signs of discomfort.

*Toxic bait trails.* Time allowed for only limited trials of toxin-laced baits. Results showed that the method has some promise but was mostly unsuccessful. Of 15 *Anolis carolinensis* offered toxic baits, only one died when offered a free choice among four different bait types (Tables 5, S5). It was not possible to identify which bait provided this single success, but all animals offered only red corn syrup survived and showed no signs of toxification. *Hemidactylus frenatus* seemed more prone to take baits, with eight of 20 becoming toxified and two dying, but the remaining 12 animals were alive and well at the end of trials and showed no signs of ill health. Of the six animals that became toxified but did not die, one recovered by 45 h, and the remaining five were still moribund at the end of the trials, being either inert but breathing or able to slowly move.

## Discussion

We obtained two important results with respect to identifying an effective toxicant for small lizards. First, acetaminophen was infeasible for controlling small lizards, even though it has proven effective for controlling brown treesnakes (Savarie et al. 2000, 2001; Clark and Savarie 2012) and proven lethal against other large lizards and snakes (Mauldin and Savarie 2010; Avery et al. 2011). The reasons for this are two-fold. Dosages needed are large relative to the solubility of the toxin in aqueous solutions (14 mg/ml), and it proved impossible to suspend sufficient amounts of acetaminophen in liquid baits for delivery to the subjects because the high concentrations needed formed a thick slurry that could not be reliably pipetted. Further, acetaminophen is very bitter to humans, and, if lizards have similar taste receptors, the bitterness of any suspension of acetaminophen is likely to discourage sufficient consumption to induce toxicity. As such, acetaminophen is clearly infeasible for controlling small lizards, and we suspended tests of the compound before identifying a reliable LD100. When acetaminophen has proven effective against squamates, the target species were sufficiently large that acetaminophen capsules could be placed in baits that were consumed whole (Savarie et al 2000, 2001; Mauldin and Savarie 2010), thereby avoiding problems with solubility and taste. Even so, other large lizards tested have required such high dosages of acetaminophen to achieve lethality that the toxin was deemed impractical for field use (Avery et al. 2011).

Secondly, we found alpha-chloralose to be toxic for the lizards tested in this study at concentrations up to ten times less than those tested yet found wanting for acetaminophen (Table 4). Further, the compound proved effective in less than two days (and usually within a day), and it is not bitter, at least to humans. Like acetaminophen, alpha-chloralose has a low solubility in water (4.5 mg/ml), requiring its use as a suspension, but the lesser amounts of compound needed for toxicity alleviate this problem, though consistent suspension in a liquid matrix remains a problem inasmuch as the compound tends to settle out in corn syrup with time. This difficulty of obtaining a reliable suspension, even within a solution as viscous as corn syrup (2000–3000 cP = 2000–3000 mPa·s), suggests that an even more viscous solution or pastes could be more feasible delivery systems. However, in this study we were unable to develop such alternatives.

Identification of an effective toxin is only one requirement for developing a successful control tool for small invasive lizard species. Also needed is an effective bait, and our results were less successful in this regard though suggestive that a feasible bait could be developed for some species with further work. Our results indicated that baits featuring red color with or without vanilla scent were generally more attractive than other baits for *A. carolinensis*, although yellow paste was also preferred over orange paste (Table 1). Similarly, white corn syrup with vanilla scent

or red corn syrup alone were most attractive for *H. frenatus* (Table 2). However, results were overall rather variable among the many sets of binary trials, and measured consumption differences were small overall. There are two likely reasons for these sometimes-inconsistent results. First, the lizards used in this study were chosen because of severity of their ecological impacts and because they were readily available in sufficient quantities to meet test demands. However, although many close relatives of these two species are well known to eat fruits, nectar, and/or human-made sweets (jams, candies), we can find only a single reference to *A. carolinensis* doing so (Hodge et al. 2003; Valido and Olesen 2019) and no information in the literature pointing to *H. frenatus* doing so (Valido and Olesen 2019). Consequently, our two primary test species may not be as attracted to these baits as some of their relatives would be, and this would, of course, reduce our success in identifying satisfactory baits. Secondly, we became concerned through the course of our experiments that the design we followed for determining consumption was not as reliable as we initially expected. We became convinced that our initial use of a balance with a precision of 0.01 g to measure consumption differences was insufficiently precise. When we corrected that deficiency during our second bout of tests by using a balance with a precision of 0.001 g, we routinely discovered that baits gained weight during our trials due to absorption of water vapor from the air, requiring us to infer bait attractiveness by determining which bait gained less weight relative to our controls. Obviously, that is not as straightforward a means of assessing bait attractiveness as one might hope for. Given that we have now identified an effective toxin for these lizards, we suggest that future bait trials would be more reliable if they used toxic baits and assessed percent lethality among test animals instead of attempting to measure differences in bait consumption. Alternatively, one could use time-lapse photography or video of lizards to analyze number of licks or amount of time spent feeding, though that would be a very time-consuming approach.

Our preliminary trials combining alpha-chloralose with a selection of the most attractive baits to assess whether toxic baits could conceivably succeed as a means of controlling these lizards met with limited success. For each species, there were a few animals that consumed sufficient bait to die, but that number was too small to be of practical use in a control or eradication program (Table 5). More often, there was no sign of toxification in the lizards—suggesting they did not consume the baits—or lizards showed early signs of toxification but recovered later. More specifically, 1 of 15 *A. carolinensis* tested died, and the remainder showed no signs of toxification, suggesting they did not consume the baits at all. Two of 20 *H. frenatus* died, and another six were initially toxified but had failed to die by the time trials were terminated (Table 5). These differences between the species accord with our impressions from the bait trials: *H. frenatus* seemed more inclined to consume baits than did *A. carolinensis*, and it may

be that this *Anolis* species is not particularly attracted to sweet food items. Initial toxification of *H. frenatus* confirmed that a significant portion of the test sample was in fact attracted to and consumed offered baits. However, they clearly failed to consume sufficient bait to die. Development of a more viscous bait matrix that allowed for a more concentrated suspension of alpha-chloralose might prove more effective for these geckos by reducing the amount of consumption required to prove lethal.

It will be obvious that the results we present here are initial and only suggest the broad outlines for developing effective tools for field use. Further work is needed, and it is worth noting that, for comparison, the U.S. Government has been investigating means to control brown treesnakes on Guam since the mid-1990s, and both artificial baits and an automated system to deploy acetaminophen-laced mouse baits by helicopter are only now nearing completion (e.g., Kimball et al. 2016; Siers et al. 2019, 2020) despite dead neonatal mice and acetaminophen having been known to be the best attractant and toxicant since 1997 and 2000, respectively (Shivik and Clark 1997; Savarie et al. 2000, 2001). Three lines of research provide the next logical steps in advancing toxin development for small-lizard control. First, bait effectiveness likely varies among lizard taxa, and further work is needed to identify those baits (substances, colors, odors) most attractive to different taxa, including those species tested here. Second, better bait matrices need to be developed. In particular, we found the failure of corn syrup to keep alpha-chloralose (or acetaminophen) in uniform suspension to be a problem that would compromise its use in any field setting. Both liquid and paste baits should be developed because each may prove useful under different conditions: with feeding stations holding liquid baits being perhaps more easily deployed and visually obvious (like hummingbird feeders) in natural habitats, and pastes more easily deployed around buildings or in cargo containers. Both with respect to attractants and bait matrices, development of effective bait-delivery systems will likely best proceed in collaboration between invasive-species biologists and formulation chemists, an option unavailable during this study. Lastly, it could prove useful to identify one or more additional toxicants that are as effective as alpha-chloralose yet with a higher aqueous solubility. Availability of a toxicant with fewer solubility problems would allow greater freedom in bait formulation.

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## Ethics and Permits

This study was approved as NWRC Protocol QA-1747 by the Institutional Animal Care and Use Committee of the USDA National Wildlife Research Center, Fort Collins, Colorado. All State of Hawaii and private collection permits and rights of access were adhered to.

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**Supplementary material**

The following supplementary material is available for this article:

**Table S1.** Raw data for bait trials with *Anolis carolinensis*.
**Table S2.** Raw data for bait trials with *Hemidactylus frenatus*.
**Table S3.** Raw data for bait trials with *Lampropholis delicata*.
**Table S4.** Toxicant trials for *A. carolinensis*, *H. frenatus*, and *L. delicata*.
**Table S5.** Toxic bait trials for *A. carolinensis* and *H. frenatus*.
**Table S6.** Differences in bait consumption and weight change of controls in bait trials with *A. carolinensis*.
**Table S7.** Differences in bait consumption and weight change of controls in bait trials with *H. frenatus*.
**Table S8.** Differences in bait consumption and weight change of controls in bait trials with *L. delicata*.

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