FINAL REPORT

Title:

Field evaluations of non-toxic unadulterated dead neonatal mice (DNM), freeze-dried DNM, and dehydrated DNM in brown treesnake bait stations and live traps.

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ABSTRACT

The two primary objectives of this study were to evaluate freeze-dried dead neonatal mice (fdDNM) and dehydrated dead neonatal mice (dDNM) as bait and lure attractants for brown treesnakes (BTS). Unadulterated dead neonatal mice (uDNM) are used as the bait matrix for acetaminophen which is a U.S. EPA registered oral toxicant for BTS. Live mice are the lure attractants for capturing snakes in live traps. Shipping of frozen uDNM and maintaining live mice is expensive and practical replacements for each would be of benefit for the operational control of BTS. Snake consumption (bait take) of 3 dead neonatal mouse treatments, uDNM (the positive control), fdDNM, and dDNM, was evaluated under field conditions on Guam. Consumption was similar for each of the 3 bait types and ranged from 48%-96%. The uDNM baits decomposed quickly in the field and lost 50% of their body mass between 1-2 days; 50% loss of body mass for the fdDNM and dDNM was between 4-5 days. Increased longevity in the field of either fdDNM or dDNM did not result in increased bait acceptance. Snake captures in traps with the live mouse far exceeded those of the other treatments as follows: live mouse – 95 BTS captured; uDNM – 10; fdDNM – 1; and dDNM – 2. Consumption of beef baits treated with decomposition products from DNM that had “aged” for 48 h under ambient field conditions was similar to uDNM, fdDNM, and dDNM. Future studies will evaluate bait consumption of synthetic baits treated with decomposition products from DNM.

INTRODUCTION

Brown treesnakes (*Boiga irregularis*, BTS) are an invasive species on Guam and cause extensive ecological and economic damage (Savidge 1987; Fritts et al. 1987; Rodda and Fritts 1992a). Operational efforts to control the snake population and to deter snake dispersal to Hawaii, other Pacific islands, and the U.S. mainland, include the use of live traps, search of cargo facilities with detector dogs, and spotlighting of fences (Hall 1996, Engeman and Vice 2001). Traps with live mouse lures (Engeman et al. 1998, Linnell et al. 1998, Rodda and Fritts 1992b, U.S. Department of Agriculture 1996) capture the majority of snakes (D. S. Vice, pers. comm.). More recently, dead neonatal mice (DNM) treated with 80 mg acetaminophen were found to be effective for reducing brown treesnake populations on Guam (Savarie et al. 2001), and acetaminophen is registered as an oral toxicant for brown treesnake control by the U.S. Environmental Protection Agency (Reg. No. 56228-34). DNM serve as both an attractant (lure) and bait matrix for BTS.

Despite the success with DNM and live mice, there are logistical and economic reasons for alternate lures (Fritts et al. 1989; Shivik and Clark 1999). Frozen DNM are air freighted and stored frozen before use. A missed airflight connection or power outage could result in a thawed, decomposed, ruined shipment. DNM also putrefy after 3-4 days in the field and become less acceptable as baits. Live mice require a water source from potatoes which have to be shipped to Guam and there is also the expense of preparing wax grain blocks as a food source. Additionally, there is the expense for housing and maintaining live mice before deployment in the live traps. Freeze-dried and dehydrated DNM have the potential for storage at ambient conditions which would be less expensive and more convenient and could be “stockpiled” in anticipation for operational programs.

The primary objectives of this field study were to evaluate freeze-dried and dehydrated DNM as bait and trap lures for brown treesnakes, and to compare consumption of beef treated with dead mouse odor to that of untreated DNM and beef by snakes.

METHODS AND MATERIALS

Four field experiments were conducted on Guam on Andersen Air Force Base near Tarague Beach and the Sanitary Landfill area, and Navy Radio Barrigada during 2006 and 2007. PVC bait tubes and live traps were cleaned with mild chlorine bleach solution (1:50 chlorine-water) and rinsed with water before use. Frozen unadulterated dead neonatal mice (DNM, 4-7 g) were purchased from Essex Co., P.O. Box 581, Blum, Texas 76627 USA. Batches of intact DNM were prepared either in a freeze dry system (Labconco Model 77540, Freezone® 12 L, Kansas City, Missouri 64132 USA) or a dehydrator (Model FD-1008P, Alternative Pioneering Systems, Inc., Chaska, Minnesota 55318 USA). The freeze dry system was programmed for durations totaling 140 -176 h that cycled from -10ºC to -5ºC and finished at 0ºC. Moisture loss for the freeze-dried DNM ranged from 56%-62%. The dehydrator operated at 70ºC for 24 h and moisture loss for the dehydrated DNM ranged from 57%-69%. Freeze-dried DNM and dehydrated DNM were vacuumed sealed (Jarden Corp., San Francisco 94119 USA) in separate bags and frozen. All DNM treatments were air freighted frozen to Guam, stored frozen, and thawed before application in the field.

Experiment 1. Bait Evaluations with Freeze-dried and Dehydrated DNM

Three bait matrices were evaluated: unadulterated DNM (uDNM), freeze-dried DNM (fdDNM), and dehydrated DNM (dDNM). uDNM are the bait matrix currently used operationally for delivery of acetaminophen (Savarie et al. 2001) and were the positive standard. The 3 bait matrices were tested in PVC tubes (7.6 cm diameter x 30.5 cm long, bottom ends were open and tops were closed by plastic caps with 0.3 cm diameter holes). Tubes were positioned at 45º and hung about 1.5 m high in vegetation along the forest perimeter adjacent to roads and trails at 20 m intervals. Ten baits for each of the 3 bait matrices were randomly placed per transect (n=30 bait stations per transect) and 3 transects were tested. Bait take was recorded daily for 6 days. Baits taken by ants and maggots (as evidenced by skeletons) were deleted from the data set. For example, a skeleton found on Day 3 was deleted from the total number of baits available to snakes on Day 4. Transects were moved to a new location after Day 6. Five baits in each transect were randomly selected and monitored for about 24 h each day by video recording equipment (Supercircuits, Austin, Texas 78759 USA). An infrared video camera for night-time viewing and videocassette recorder (VCR) were powered by a deep-cycle 12V battery that was recharged every day. The VCR and battery were housed in a weatherproof container and video tapes (Sony T-160 VHS) were changed daily. Video tapes were scored for BTS and non-target presence.

Experiment 2. Live Trap Capture Evaluations

Four trap lures were evaluated: live mice, uDNM, fdDNM, and dDNM. Live mice are used operationally in traps and were the positive control. Traps without a lure were the negative control. The 4 trap lures were tested in USDA/Wildlife Services one piece traps with built-in chambers for housing the live mice (Engeman and Vice 2001). The 3 DNM lure types were hung by 0.1 mm diameter stainless steel wire inside the chamber. Traps were hung about 1.5 m high in vegetation along the forest perimeter at 20 m intervals adjacent to roads and trails. Ten traps for each of the 4 lures, plus 10 traps without lure, were randomly placed per transect (n=50 traps per transect) and 3 transects were tested. Traps were checked daily for 6 days. After Day 6 the transect was moved to a new location. Five traps with lures in each transect were randomly selected and monitored by video camera as described above and tapes were scored for presence of BTS and non-target animals.

Experiment 3. Bait Evaluations with Beef treated with Dead Mouse Odor

Five bait matrices (uDNM, fdDNM, dDNM, untreated beef [ubeef; U.S. Chill Beef Bottom Round Roast], and beef treated with decomposition products of uDNM [trtbeef]) were evaluated. Baits were placed in horizontal PVC tubes (5.1 cm diameter x 30.5 cm long) with 0.64 cm diameter bolts bisecting the ends of the tubes. The tubes were hung about 1.5 m high in vegetation along the forest perimeter at 20 m intervals adjacent to roads and trails.

DNM decomposition products were obtained by "aging" uDNM for 48 h under ambient field conditions in snake-proof enclosures (10.2 cm diameter x 30.5 cm long PVC tubes with ends closed by fine nylon mesh) hung in vegetation. Treated beef was prepared by placing 10 pieces of beef that approximated the size of DNM with 5 aged DNM in a closed glass jar and mixing for 1 h using a Wheaton benchtop roller. Ten baits for each of the 5 bait matrices were randomly placed per transect (n=50 bait stations per transect). Three transects were run. Bait take was recorded daily for 4 days.

Experiment 4. Decomposition of DNM at Ambient Temperature and Humidity

Three bait matrices, uDNM, fdDNM, and dDNM, were evaluated in PVC tubes (7.6 cm diameter x 30.5 cm long) hung horizontally about 1.5 m high in vegetation along the forest perimeter at 20 meter intervals. Both ends of the tubes were closed with 7 mm wire mesh that excluded entry of snakes but not flies and ants. Five baits for each of the 3 bait matrices were randomly placed in one transect (n= 15 tubes). Mass (g) of baits was recorded daily for 7 days.

RESULTS AND DISCUSSION

Experiment 1. Bait Evaluations with Freeze-dried and Dehydrated DNM

The cumulative bait take of unadulterated dead neonatal mice (uDNM), freeze-dried DNM (fdDNM), and dehydrated DNM (dDNM) by snakes for each of the 3 transects are summarized in Figure 1. Mean percent cumulative bait-take by snakes did not differ among DNM bait types over the 6 day observation period (repeated-measures ANOVA; $F_{2,6} = 2.43$, $P = 0.17$). The mean 6 day cumulative bait-take of uDNM, fdDNM, and dDNM was 96.3%, 54.7%, and 47.8%, respectively. Overall, maximum bait take for each of the 3 DNM bait treatments was on Days 2 and 3. For uDNM and fdDNM, no baits were consumed during Days 4-6; and only 3 dDNM baits were consumed during Days 4-6. The apparent increase in bait consumption with time is mostly an artifact due to baits taken by ants and maggots.

The cumulative bait-take of uDNM, fdDNM, and dDNM by ants and maggots for each of the 3 transects are summarized in Figure 2. Mean cumulative bait take by ants and maggots did not differ among DNM bait types over the 6 day observation period (repeated-measures ANOVA; $F_{2,6} = 0.45$, $P = 0.65$). The majority of baits were taken between Days 3 and 4. On one of the 3 dDNM transects, snakes did not take dDNM and 9 of 10 baits (90%) applied were taken by ants and maggots by Day 3. From the total of 30 baits each applied for uDNM, fdDNM, and dDNM, ants and maggots took 15, 12, and 18, respectively.

Six (6) uDNM, 5 fdDNM, and 4 dDNM bait stations were monitored by video camera and video tapes scored for activity. Except for ants and flies, no non-target animals such as crabs or rats made contact with the bait stations. Three of the 6 uDNM were taken but only one tape shows a brown treesnake with the uDNM in its mouth. For the other 2 uDNM that were taken, one snake apparently consumed the uDNM inside the tube and one snake was in the field of view but the camera malfunctioned before the snake presumably entered the bait station and took the bait. Snakes did not take any of the 5 fdDNM baits. Two of the 4 dDNM baits were taken. In both cases a snake was observed inside each of the bait stations but no dDNM was observed in the mouth when the snakes exited the bait stations. Presumably each of the 2 snakes consumed the dDNM inside the bait stations.

Experiment 2. Live Trap Capture Evaluations.

Snake captures in the 5 live trap treatments (empty trap, live mice, uDNM, fdDNM, and dDNM) are summarized in Table 1. No snakes were captured in empty traps. Total snake captures with live mice was 9.5 times greater than uDNM, 95 times greater than fdDNM, and 48 times greater than dDNM. These results show that the 3 dead mouse treatments would not have application as attractant lures for live capture of snakes.

Experiment 3. Bait Evaluations with Beef Treated with Dead Mouse Decomposition Products.

The cumulative bait take of uDNM, fdDNM, dDNM, ubeef, and trtbeef by snakes are summarized in Figure 3. Mean cumulative bait take differed among bait types (Repeated-measures ANOVA; $F_{4,10} = 15.3$, $P = 0.0003$). As expected, bait take increased over the four day observation period (repeated-measures ANOVA; $F_{3,10} = 5.5$, $P = 0.004$). The interaction term was not significant (repeated-measures ANOVA; $F_{12,30} = 1.3$, $P = 0.29$)

There is a dramatic increase in beef consumption when beef is treated with the decomposition products from 48 h “aged” mice. The 4-day cumulative bait take of untreated beef and treated beef is 7% and 67%, respectively, and 63% of the treated beef is consumed on Day 1 (Figure 3). Bait take by ants and maggots was low with the following numbers of baits taken: uDNM – 1, fdDNM – 5, dDNM – 3, ubeef – 2, and trtbeef – 1.

Experiment 4. Temporal Decomposition of DNM at Ambient Temperature and Humidity

At the beginning of the experiment on Day 0, the average mass of uDNM was higher than those of the other two groups and the average mass of fdDNM was higher than that of dDNM (Figure 4). There was a significant interaction between DNM treatment and number of days in the field (repeated-measures ANOVA, $F_{14,84} = 47.2$, $P < 0.0001$). From inspection of Figure 4 it is apparent that at least part of the observed significance is due to inclusion of the uDNM group; on Day 0 and Day 7, uDNM mean bait masses were higher and lower than those of the fdDNM and dDNM groups, respectively. To investigate whether there might also be an interaction between the fdDNM and dDNM groups, a second analysis was conducted using just those two groups. In this analysis, the interaction between DNM treatment and number of days in the field was also significant (repeated-measures ANOVA, $F_{7,56} = 3.5$, $P = 0.004$). From inspection of Figure 4, it is apparent that average bait masses in the fdDNM group were decreasing faster that those in the dDNM group.

Another indication of the difference between uDNM and the other 2 treatments is the time when body mass decreased by 50%. For uDNM it was between 1-2 days and for both fdDNM and dDNM it was between 4-5 days. Although fdDNM and dDNM appeared to retain their physical characteristics longer than uDNM, bait consumption for each of these 3 DNM treatments was similar.

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TABLES

Table 1. Daily snake capture for each of 5 trap treatments for 6 exposure days in the field (n=60 trap nights/trap treatment/transect).

Transect No.	Trap treatment*	Day						Total captures	Captures/ trap night
		1	2	3	4	5	6		
1	ET	0	0	0	0	0	0	0	0
	LM	4	5	5	4	5	7	30	0.50
	uDNM	0	0	0	0	0	0	0	0
	fdDNM	0	0	0	0	0	0	0	0
	dDNM	0	0	0	0	0	0	0	0
2	ET	0	0	0	0	0	0	0	0
	LM	12	4	7	8	9	4	44	0.73
	uDNM	3	3	1	0	0	0	7	0.12
	fdDNM	0	0	0	0	0	0	0	0
	dDNM	0	0	0	1	0	0	1	0.02
3	ET	0	0	0	0	0	0	0	0
	LM	1	3	2	5	7	3	21	0.35
	uDNM	0	3	0	0	0	0	3	0.05
	fdDNM	0	1	0	0	0	0	1	0.02
	dDNM	0	1	0	0	0	0	1	0.02
Totals	ET	0	0	0	0	0	0	0	0
	LM	17	12	14	17	21	14	95	0.53
	uDNM	3	6	1	0	0	0	10	0.06
	fdDNM	0	1	0	0	0	0	1	0.006
	dDNM	0	1	0	1	0	0	2	0.01

* ET= empty trap
LM= live mouse
uDNM= unadulterated dead neonatal mice (DNM)
fdDNM= freeze dried DNM
dDNM= dehydrated DNM

FIGURES

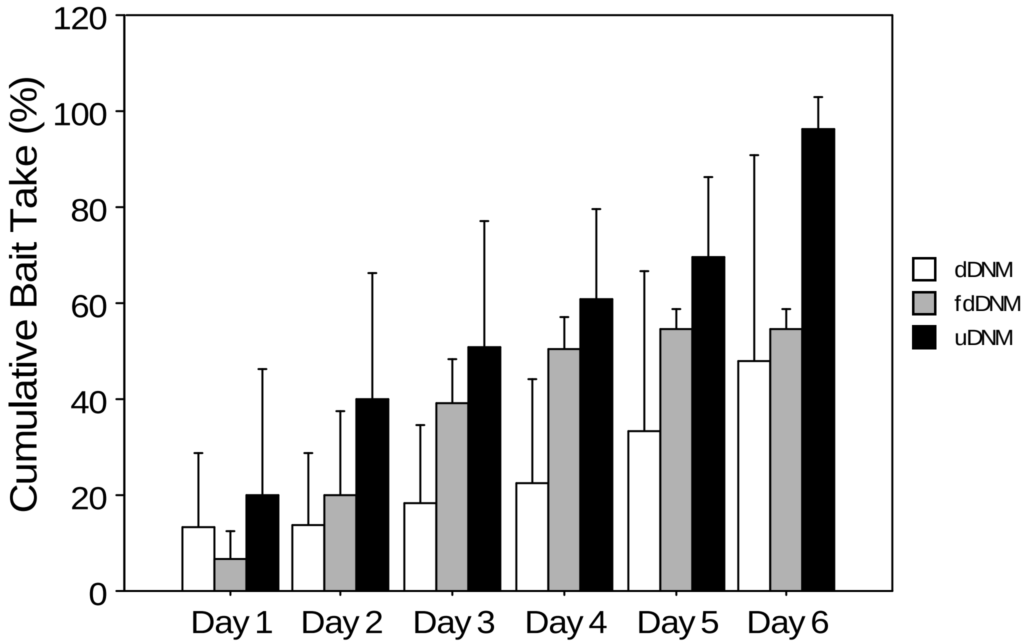

Figure 1. Cumulative bait take (mean ± SD) by brown treesnakes in experiment 1 for unadulterated dead neonatal mice (uDNM), freeze-dried (fdDNM), and dehydrated (dDNM). Three transects were run with 10 baits per bait type per transect (n= 30 of each bait type evaluated).

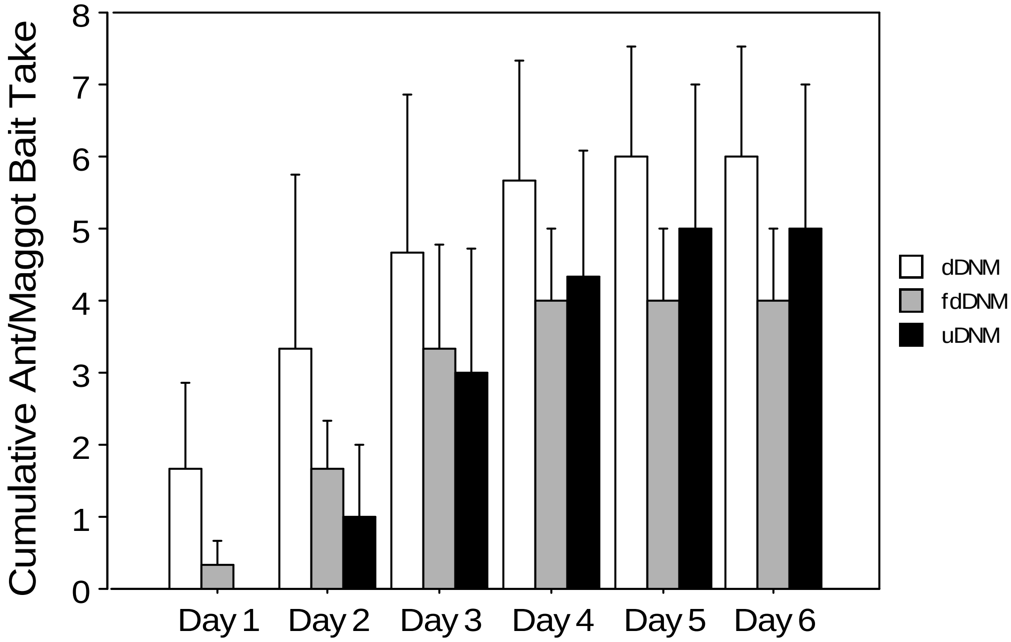

Figure 2. Cumulative bait take (mean ± SD) by ants and maggots in experiment 1 for unadulterated dead neonatal mice (uDNM), freeze-dried (fdDNM), and dehydrated (dDNM). Three transects were run with 10 baits per bait type per transect (n= 30 of each bait type evaluated).

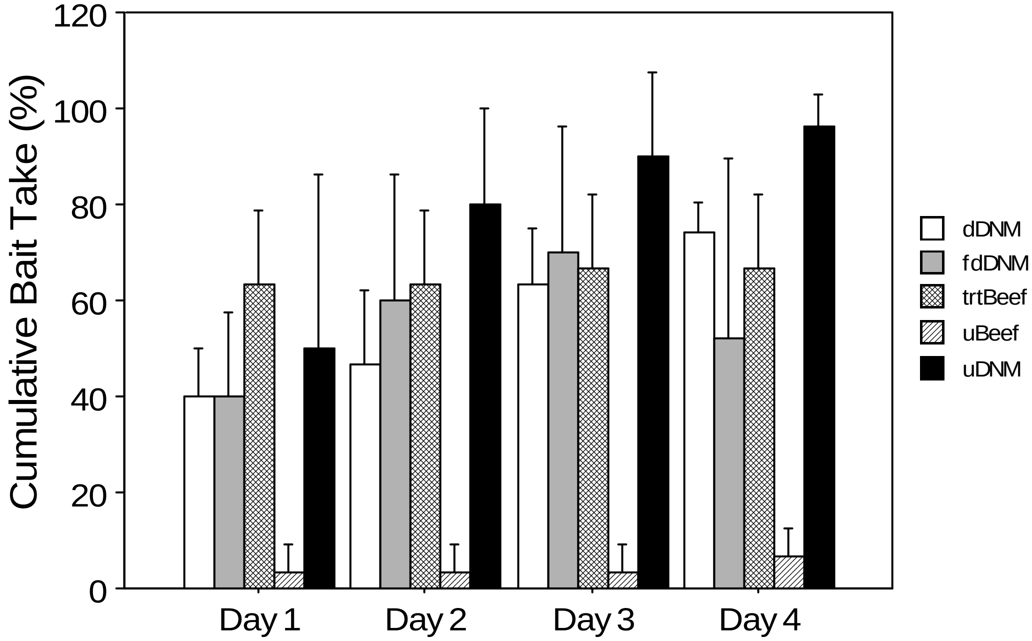

Fig. 3. The cumulative bait take (mean ± SD) by brown treesnakes in experiment 3 for unadulterated dead neonatal mice (uDNM), freeze-dried (fdDNM), dehydrated (dDNM), unadulterated beef (uBeef), and treated beef (trtBeef) as a function of time. Beef was treated with the decomposition products of dead mice that had “aged” under field conditions for 48 h. Three transects were run with 10 baits per bait type per transect (n= 30 of each bait type evaluated).

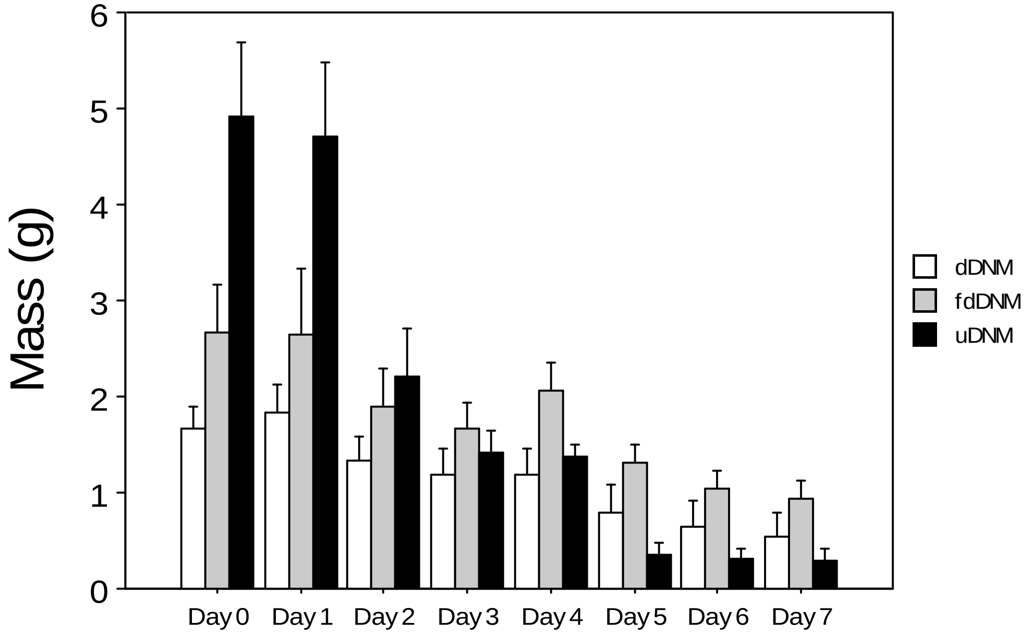

Figure 4. Body mass (mean ± SD) for unadulterated dead neonatal mice (uDNM), freeze-dried (fdDNM), and dehydrated (dDNM) as a function of decomposition in experiment 4. Five baits for each of the 3 bait types were randomly placed in 1 transect and exposed to ambient temperature and humidity conditions for 7 days.