

## IMPORTANCE OF BACTERIAL DECOMPOSITION AND CARRION SUBSTRATE TO FORAGING BROWN TREESNAKES

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**Abstract**—Brown treesnakes are an invasive species to the island of Guam that have caused extensive ecological and economic damage. Efforts to control the snake population have included trapping using live mouse lures, but for logistical and economic reasons a synthetic lure is needed. When searching for live food, brown treesnakes use both visual and odor cues. However, when searching for carrion, odor cues are sufficient. Attempts to develop synthetic lures based on chemical reconstruction of the complex carrion odor have not succeeded. We provide evidence that a microbial–substrate interaction is important for bait take by brown treesnakes. Microbial cultures taken from mouse carrion indicate that *Enterobacter agglomerans* is the predominant bacterium, and field tests suggest that this organism may be important to odor production that attracts brown treesnakes. This information may prove useful in the development of microbial-based biological reactors that could be formulated to produce a continuous stream of odor of sufficient complexity so as to be attractive to foraging snakes.

**Key Words**—Bacteria, bait, *Boiga irregularis*, brown treesnake, carcass, carrion, decomposition, *Enterobacter agglomerans*, lure, odor, rot

### INTRODUCTION

The brown treesnake (*Boiga irregularis*) was accidentally introduced to Guam in the 1940s or early 1950s (Savidge, 1987; Rodda et al., 1992). Since then the island's snake population has increased dramatically, at times reaching densities of 50–100 snakes/ha (Rodda et al., 1992). The ecological and economic effect of the

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snake has been devastating, ranging from power outages to extirpation of avifauna and herpetofauna (Savidge, 1984, 1987; Fritts et al., 1987; Conry, 1988; Engbring and Fritts, 1988; Rodda and Fritz, 1992a). Guam's importance as a shipping hub makes the spread of brown treesnakes from Guam a threat to other sensitive Pacific Island ecosystems (e.g., Fritts, 1988). As part of a containment policy, the U.S. Department of Agriculture employs a variety of methods to reduce the likelihood that brown treesnakes will be exported inadvertently from Guam. These methods include trapping, search dogs, and hand removal of snakes from fences during spotlight searches (U.S. Department of Agriculture, 1996).

Trapping is a highly effective method for removing snakes from an area (Rodda and Fritts, 1992b; U.S. Department of Agriculture, 1996; Engeman et al., 1998). However, since live mice are used as lures, this method involves substantial logistical effort in field and laboratory maintenance, which in turn limits the number of traps that can be employed, especially for large-scale operations (Fritts et al., 1989; Shivik and Clark, 1999a). As a consequence, considerable research effort has been invested in searching for artificial lures that would reduce reliance on live mice, and hence reduce the labor needed to carry out an effective control operation using traps.

For live prey, brown treesnakes attend to both visual and olfactory cues (Chiszar et al., 1988, 1993, 1997a; Fritts et al., 1989; Shivik, 1998; Shivik and Clark, 1999a). Presentation of a combination of cues from these modalities is necessary to produce the most intense investigatory and foraging behavior in brown treesnakes (Chiszar et al., 1997b; Shivik, 1998; Lindberg et al., 2000). Somewhat surprisingly, brown treesnakes also actively forage for carrion in the field, and the carrion's effectiveness for capture is only slightly less than that seen for live mice (Shivik, 1998; Shivik and Clark, 1997, 1999a,b; Shivik et al., 2000). However, most notable was the observation that chemosensory cues were predominantly critical to brown treesnakes when foraging for carrion. Thus, the potential for the synthesis of an inanimate chemical lure exists. Unfortunately, attempts to reconstruct an effective chemical lure based on the chemical analysis of mouse carrion has proved difficult and ineffective because of the chemical complexity of the carrion odor and the snakes' lack of a reaction to imperfect chemosensory cues (Clark, 1997; Shivik, 1998; Shivik and Clark, 1999a). In this study, we set out to better understand the source and nature of the attractive odor of carrion that would be helpful in the development of a bioreactor that would generate salient odors. Because the odor of carrion decomposition is largely a result of a substrate-bacterial interaction, we hypothesized that the most attractive odors to brown treesnakes would be generated from the surface of mice. We reasoned that knowledge about the appropriate nutrient substrate and the bacteria responsible for decomposition would be helpful in guiding us to construct biological (microbial) reactors.

## METHODS AND MATERIALS

We conducted experiments in the Conventional Weapons Storage Area on Andersen Air Base near Tarague and Haputo beaches, Guam, in May and June 2000. The disappearance of dead neonatal mice (DNM) from PVC tubes (i.e., bait take) was used as an index of acceptance of carrion by snakes. Analysis of over 1000 hr of video tape indicates that brown treesnakes are almost exclusively the sole vertebrate responsible for the disappearance of baits (Clark, personal observation).

Prior to an experiment PVC tubes ( $10.1 \times 30.5$  cm) were cleaned with mild chlorine bleach solution (1:100 chlorine-water) and rinsed with water. Patches of vegetation where PVC tubes were placed were systematically divided by a grid of roads. These patches of vegetation were approximately 6–12 ha, depending upon the spacing of roads (Savarie et al., 2001). Following cleansing, PVC tubes were suspended from strings at heights of 1.5–2 m in shrubs and trees and linearly placed along the forest edge adjacent to roads at 20-m intervals. When multiple transects were set out on the same day, the minimum distance between transects was 50 m, separated by road. Previous studies showed that the probability of snakes traveling this or longer distances in a given night was low (Savarie et al., 2001), hence transects could be considered independent experimental units.

Each transect consisted of  $k \cdot r$  PVC tubes, where  $k$  was the number of treatment levels and  $r$  was the number of blocks within a transect. The number of blocks within transects was constant across all experiments ( $r = 10$ ). The  $k$  treatment levels were randomly ordered within each block,  $r$ , such that each of the treatment levels was represented once within each block. We used transect as the statistical experimental unit. For each treatment level,  $k$ , the dependent variable was calculated as the proportion of baits missing, i.e., the number of baits missing divided by  $r = 10$ . Thus, the number of replicates for comparisons among treatment levels,  $k$ , for any given experiment corresponded to the number of transects,  $N$ .

Our experiments addressed three broad biological questions. In the first set of experiments we evaluated how the stage of decomposition of dead mice affected bait take by snakes. In a second set of experiments we evaluated how different parts of decomposing mice influenced bait take by snakes. In a third set of experiments we evaluated whether microbial degradation of carrion affected bait take by snakes.

*Set 1*

*Experiment 1: Importance of Decomposition of Carrion for Lure Attractiveness.* The objective of this experiment was to determine the attractiveness of fresh (<24 hr old) whole dead neonatal mice relative to 1-day old (24–48 hr) whole dead neonatal mice. In the latter case, the mice were aged under natural conditions.

Subsequently, both sets of mice were placed in the field. This experiment consisted of two treatment levels ( $k = 2$ ) with  $r = 10$  blocks per transect ( $N = 4$ ), for a total of 20 PVC tubes per transect ( $k \cdot r$ ). PVC tubes were checked for presence or absence of baits after 24 hr. Following the experiment, we removed, cleaned, and placed PVC tubes in a new, isolated edge of forest in preparation for monitoring the next transect in the experiment. Patterns of bait take were analyzed using a  $t$  test, where treatment was the between-measures factor and the proportion of baits taken was the response variable (StatSoft, 1994).

### Set 2

*Experiment 2: Whole vs. Reconstituted Mouse Lures.* The objective of this experiment was to determine whether the modification of carrion was important for the attractiveness of the lure. This experiment consisted of two treatment levels ( $k = 2$ ) with  $r = 10$  blocks per transect ( $N = 7$ ), for a total of 20 PVC tubes per transect ( $k \cdot r$ ). For one treatment level we ground whole dead neonatal mice ( $N = 10$ ) in a blender and rolled the ground meat and fur into balls (DMG). The second treatment level, the control, consisted of unaltered whole dead neonatal mice (DNM) ( $N = 10$ ). PVC tubes were checked for presence or absence of baits after 24 and 48 hr. Placement, scoring, and cleaning of PVC tubes were similar to experiment 1. Bait take was analyzed using a two-way repeated-measures analysis of variance, where treatment was the between-measures factor, day was the within-measures (i.e., repeated) effect, and the proportional cumulative bait take was the response metric (Winer 1971, p. 520). Tukey's HSD test was used to determine the post-hoc differences among means for the main effect, and simple orthogonal contrasts were used to compare group means for the interaction term (StatSoft, 1994).

*Experiment 3: Attractiveness of Mouse Parts and Tofu-wrapped Pelts.* Grinding and combining mouse parts substantially reduced the attractiveness of baits to foraging brown treesnakes (experiment 2). Therefore, the objective of this experiment was to determine whether dissected, intact mouse parts were attractive baits. To separate pelts from carcasses (C), we thawed dead neonatal mice and carefully skinned them using dissecting scissors. We removed pelts at the wrist, ankle, tail bone, and rostrum; then wrapped the pelt around a piece of tofu ( $P_{\text{tof}}$ ). The tofu served as a moisture source in the field to prevent the pelt from desiccating. All three bait types (C,  $P_{\text{tof}}$ , DNM) were placed into separate plastic cups, sealed, and kept cold until transported to the field. The methods for bait placement, assigning treatment levels within transects, monitoring PVC tubes, and analysis were identical to those described in experiment 2. This experiment consisted of three treatment levels ( $k = 3$ ) with  $r = 10$  blocks per transect ( $N = 6$ ), for a total of 30 PVC tubes per transect ( $k \cdot r$ ).

*Experiment 4: Attractiveness of Mouse Parts and Cotton-wrapped Pelts.* The objective of this experiment was to determine the attractiveness of pelts with moistened cotton as a moisture source ( $P_{cot}$ ) and pelts without a moisture source ( $P$ ) relative to DNM. The methods for bait placement, assigning treatment levels within transects, monitoring PVC tubes, and analysis were identical to those described above. This experiment consisted of three treatment levels ( $k = 3$ ) with  $r = 10$  blocks per transect ( $N = 5$ ), for a total of 30 PVC tubes per transect ( $k \cdot r$ ).

### Set 3

*Experiment 5: Attractiveness of Baits Treated with Alcohol.* Because we found that pelts were taken at the same rate as DNM and that skinned carcasses were taken at lower rates (experiment 3), we limited further testing on the source of the lure odor cue to pelts in comparison to DNM. In this set of experiments, we treated pelts in one of two ways, working under the hypothesis that surface bacteria may be responsible for generating the odors to which snakes were attracted. In the first series of tests, pelts were prepared as described in experiment 3. No special treatment was afforded to nontreated pelts wrapped around tofu ( $P_{nt}$ ). A second set of pelts ( $P_{alc}$ ) was treated using alcohol. Briefly, pelts and tofu were separately soaked in 70% ethyl alcohol for 5 min prior to being wrapped together. Treated baits were placed in disinfected plastic cups, sealed for transport to the field, then placed into PVC tubes sprayed with 70% ethyl alcohol. PVC tubes and baits were again sprayed after bait placement to ensure a sanitary local environment, after which natural conditions were allowed to prevail. The methods for bait placement, assigning treatment levels within transects, monitoring PVC tubes, and analysis were identical to those described in experiment 2. This experiment consisted of three treatment levels ( $k = 3$ ) with  $r = 10$  blocks per transect ( $N = 6$ ), for a total of 30 PVC tubes per transect ( $k \cdot r$ ).

*Experiment 6: Attractiveness of Baits Treated with Bleach.* In a second set of experiments, we treated pelts with a commercial chlorine bleach (7:3,  $P_{chl}$ ) and repeated the above experiment. The methods for bait placement, assigning treatment levels within transects, monitoring PVC tubes, and analysis were identical to those described above. This experiment consisted of three treatment levels ( $k = 3$ ) with  $r = 10$  blocks per transect ( $N = 6$ ), for a total of 30 PVC tubes per transect ( $k \cdot r$ ).

We carried out two types of field laboratory microbial assays to evaluate the efficacy of the chemical treatment technique and to provide a presumptive identification of the primary bacteria cultured from the surface of mouse pelts. In the lab, we placed newly prepared baits ( $P_{alc}$ ,  $P_{chl}$ ,  $P_{nt}$ , DNM;  $N = 10$  per treatment type) onto nutrient agar plates and incubated the baits at 35°C for 24 and 48 hr, scoring the plates for presence of bacterial growth. From the field, a sample of

these same bait types was removed after the second day's evaluation, taken to the laboratory, placed on nutrient agar, incubated, and checked for bacterial growth as above. The rationale for this second evaluation was to determine if uneaten baits were more or less likely to foster bacterial growth. Finally, we streaked nutrient agar plates with swabs taken directly from the surface of DNM. Colonies of bacteria were isolated and tested for presumptive identification using Enterotube II and Oxi/Ferm tubes (BBL, Becton Dickinson).

*Experiment 7: Attractiveness of Denatured Baits.* The objective of this experiment was to determine the attractiveness of  $P_{nt}$ , of pelts that were boiled ( $P_b$ ), and of intact DNM that were boiled ( $DNM_b$ ) relative to DNM. Whole DNM and pelts were boiled for approximately 20 min to remove surface bacteria. As in previous experiments pelts were wrapped around tofu that served as a moisture source. This experiment served mainly as a pilot study for future experimentation and was one in which we assessed a nonchemical avenue of treatment while acknowledging the change in the nature of the proteins. Thus, only a single transect was used with  $N = 10$  baits per treatment level (DNM,  $DNM_b$ ,  $P_{nt}$ ,  $P_b$ ; 40 PVC tubes). The methods for bait placement, assigning treatment levels within the transect, and monitoring PVC tubes were identical to experiment 2. Comparison of treatments to DNM was done using a binomial test.

## RESULTS

### Set 1

*Experiment 1: Importance of Carrion Decomposition for Lure Attractiveness.* Carrion decomposed for two days was more attractive to brown treesnakes. There was a 90% increase in the rate at which 2-day-old whole mouse carrion was removed from PVC tubes relative to 1-day-old mouse carrion (Figure 1).

### Set 2

*Experiment 2: Whole vs. Reconstituted Mouse Lures.* Whole mouse carrion was more attractive as a bait than reconstituted ground mouse (Figure 2A). DMG was 20% as attractive to brown treesnakes as DNM. As a function of decomposition over two days (Figure 2B), there was little increase in attractiveness for DMG, and a large increase in attractiveness for DNM.

*Experiment 3: Attractiveness of Mouse Parts and Tofu-wrapped Pelts.* Snakes removed whole dead mice and mouse parts from PVC tubes at different rates (Figure 3A). The cumulative proportional bait take of skinned carcasses was 49% lower than that observed for whole dead mice. However, the cumulative bait take of whole dead mice and pelts was similar. The bait take of pelts and skinned carcasses also was similar. The day effect, where the biological substrate presumably

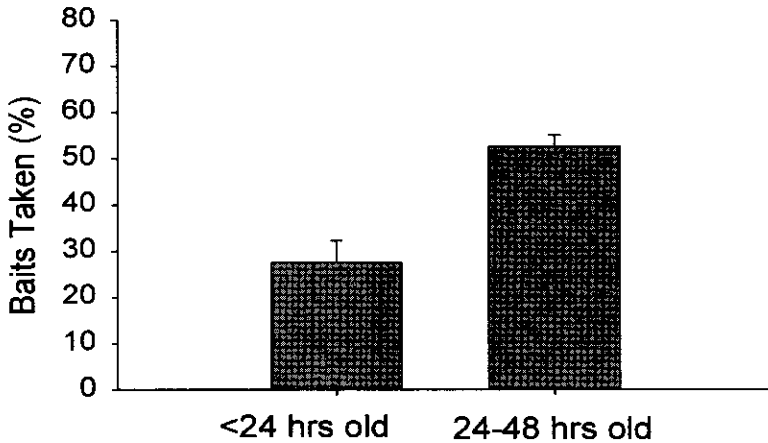


FIG. 1. Bait take (mean  $\pm$  SEM) by brown treesnakes on Guam in experiment 1 for whole dead neonatal mice as a function of bait decomposition ( $t = 21.429$ ,  $df = 6$ ,  $P = 0.004$ ). Percentage estimates were calculated using transect as the experimental unit ( $N = 4$ ) with 10 observations per bait type per transect.

becomes more decomposed on day 2 vs. day 1, indicated that bait take varied as a function of time over two days for the treatments (Figure 3B). On the first day all bait types had similar rates of take. However, by the second day the cumulative bait take was much higher for the whole dead mouse relative to skinned carcasses. Bait take for pelts on the second day was intermediate between carcasses and whole dead mice. These observations suggest that the second-day effect is partly a function of changes taking place on the surface of the mouse, i.e., skin.

*Experiment 4: Attractiveness of Mouse Parts and Cotton-wrapped Pelts.* Bait take was the same for all bait types (Figure 4A). Bait take did not vary across days as a function of treatment (Figure 4B). Thus, bait take was not substantially affected by the presence or absence of the moisture source, at least for the time frame of these experiments.

### Set 3

*Experiment 5: Attractiveness of Baits Treated with Alcohol.* Alcohol treatment diminished the attractiveness of pelts (Figure 5A). The cumulative bait take for alcohol-treated pelts was 50% lower relative to whole dead mice. The cumulative bait take for whole dead mice and nontreated pelts and that of nontreated and treated pelts were similar. The magnitude of the day effect was lowest for the alcohol-treated pelts and was proportionally similar for the whole dead mouse and nontreated pelts (Figure 5B). These observations suggest that treating the bait's

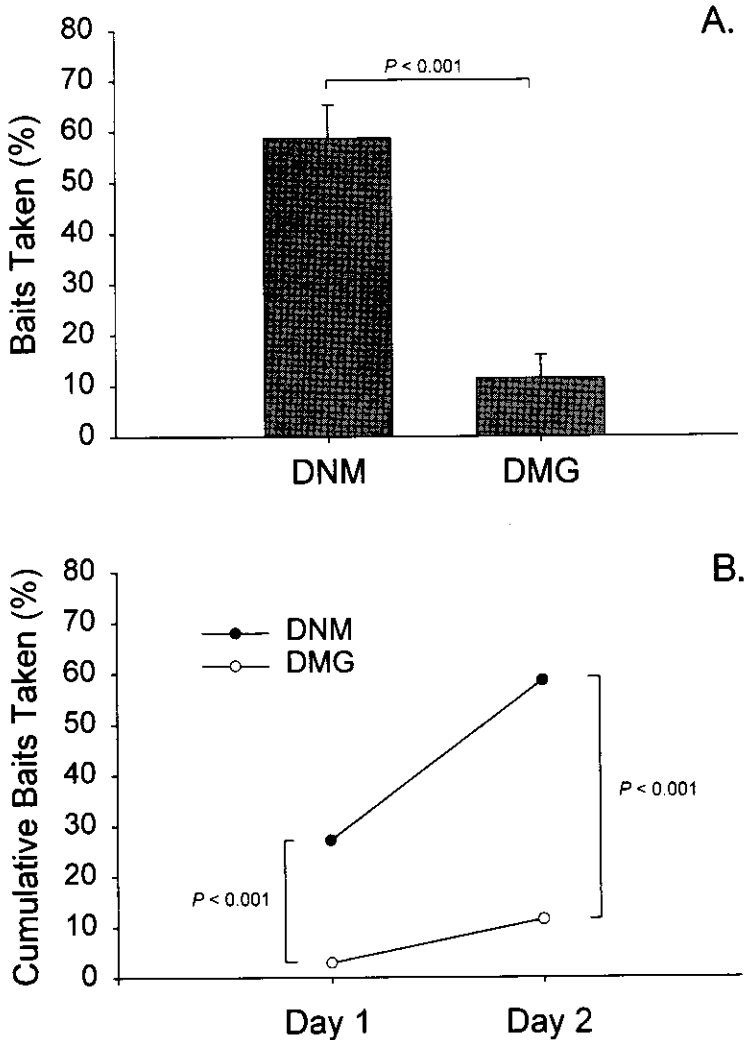


FIG. 2. (A) Bait take (mean  $\pm$  SEM) by brown treesnakes on Guam in experiment 2 for whole dead neonatal mice (DNM) and reconstituted ground mice (DMG) ( $F = 54.348$ ,  $df = 1,12$ ,  $P < 0.001$ ). Tukey's HSD comparisons and associated probability values are indicated by horizontal lines. (B) Profiles for the cumulative mean ( $\pm$ SEM) take of DNM and DMG baits as a function of time ( $F = 6.508$ ,  $df = 1,12$ ,  $P = 0.025$ ). Simple orthogonal planned contrasts and their associated probabilities are indicated by vertical lines. Percentage estimates were calculated using transect as the experimental unit ( $N = 7$ ) with 10 observations per bait type per transect.



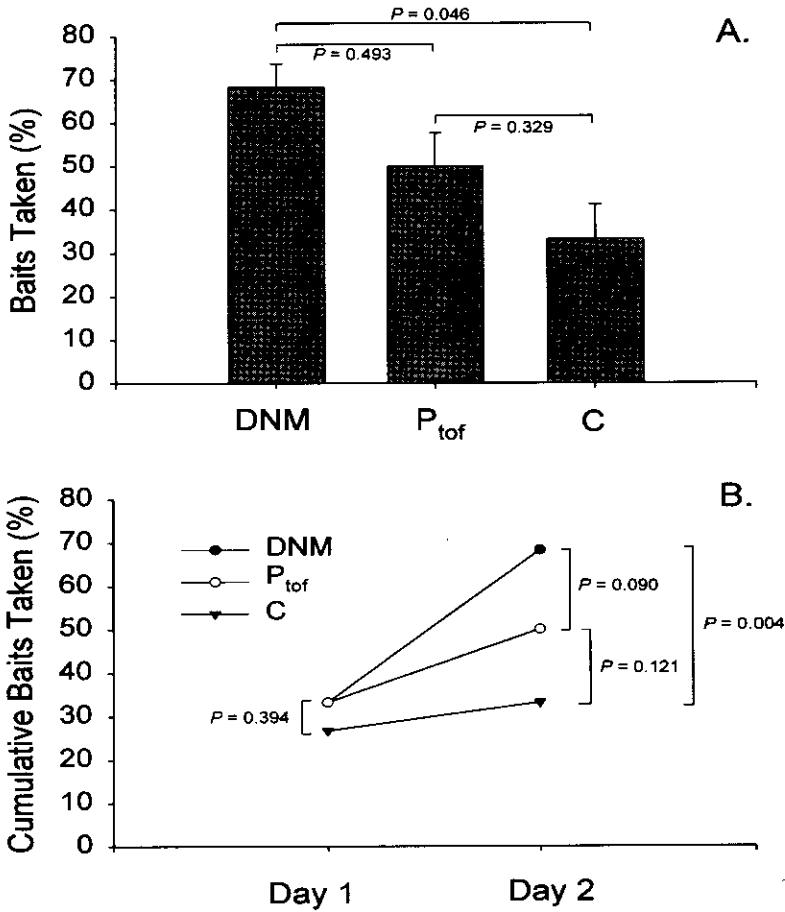


FIG. 3. (A) Bait take (mean  $\pm$  SEM) by brown treesnakes on Guam in experiment 3 for whole dead neonatal mice (DNM), mouse pelts wrapped around tofu (P<sub>tof</sub>), and mouse carcasses without skin (C) ( $F = 3.499$ ,  $df = 2,15$ ,  $P = 0.057$ ). Tofu served as a moisture source to prevent desiccation of the pelt. Tukey's HSD comparisons and associated probability values are indicated by horizontal lines. (B) Profiles for the cumulative mean ( $\pm$ SEM) bait take of DNM, P<sub>tof</sub>, and C baits as a function of time ( $F = 5.777$ ,  $df = 2,15$ ,  $P = 0.014$ ). Simple orthogonal planned contrasts and their associated probabilities are indicated by vertical lines. Percentage estimates were calculated using transect as the experimental unit ( $N = 6$ ) with 10 observations per bait type per transect.

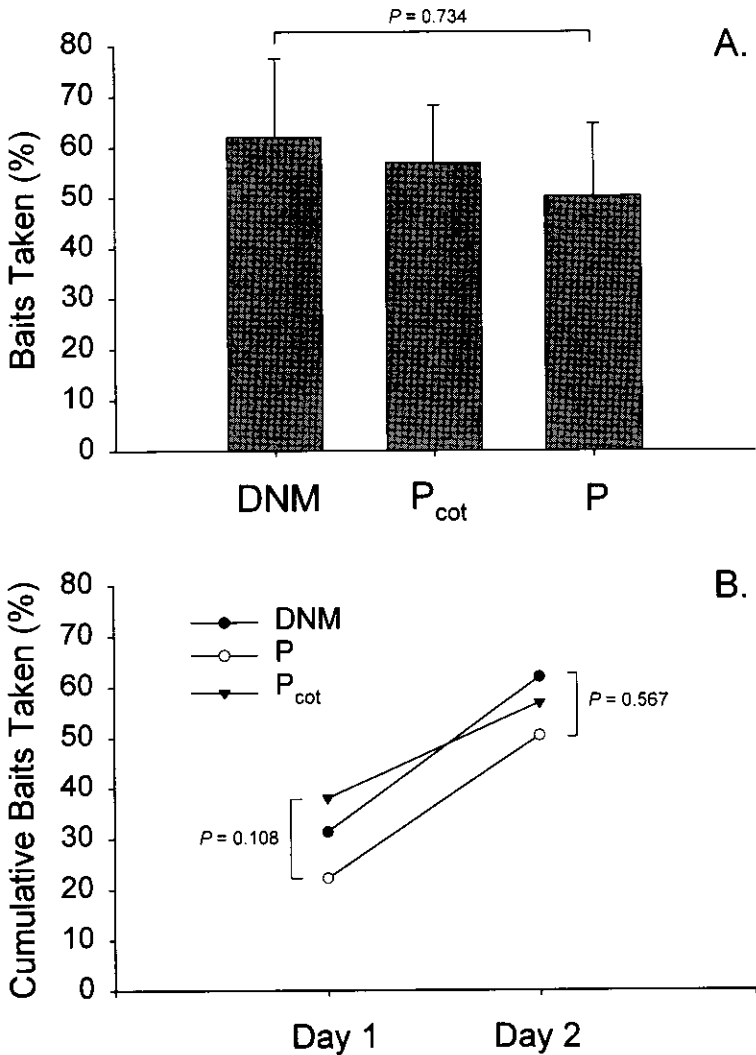


FIG. 4. (A) Bait take (mean  $\pm$  SEM) by brown treesnakes on Guam in experiment 4 for whole dead neonatal mice (DNM), mouse pelts wrapped around moistened cotton (P<sub>cot</sub>), and mouse pelts (P) ( $F = 0.416$ ,  $df = 2, 12$ ,  $P = 0.669$ ). (B) Profiles for the cumulative mean ( $\pm$ SEM) bait take of DNM, P<sub>cot</sub>, and P baits as a function of time ( $F = 0.375$ ,  $df = 2, 12$ ,  $P = 0.695$ ). Percentage estimates were calculated using transect as the experimental unit ( $N = 5$ ) with 10 observations per bait type per transect.

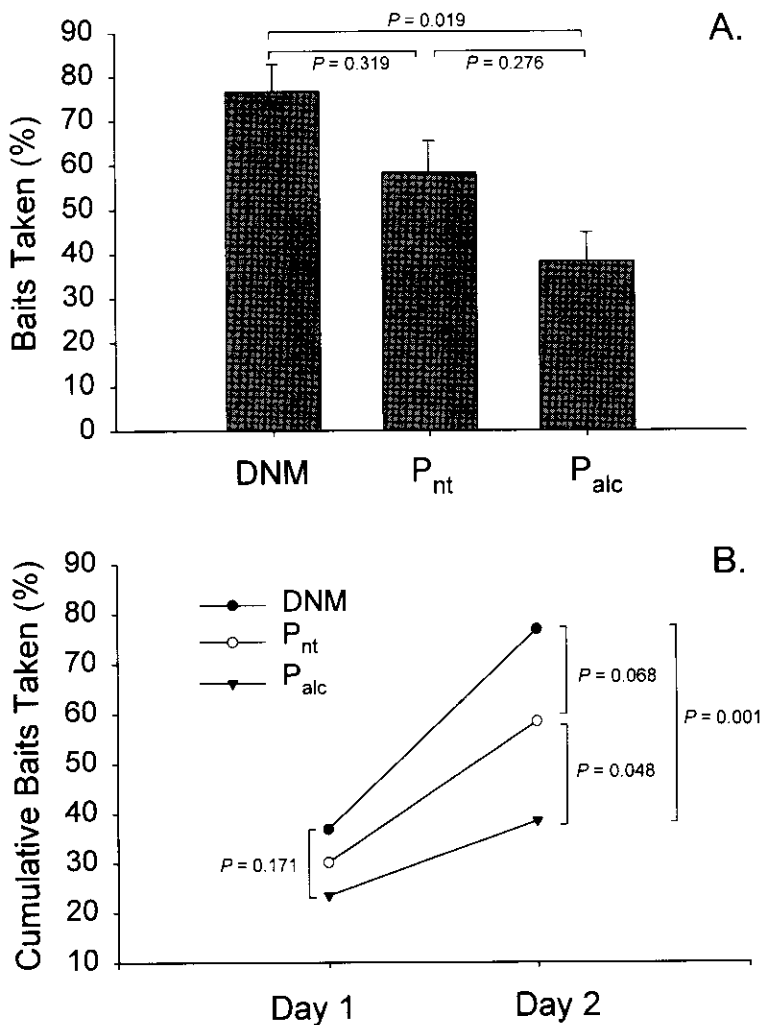


FIG. 5. (A) Bait take (mean  $\pm$  SEM) by brown treesnakes on Guam in experiment 5 for whole dead neonatal mice (DNM), nontreated mouse pelts wrapped around tofu ( $P_{nt}$ ), and alcohol-treated mouse pelts wrapped around tofu ( $P_{alc}$ ) ( $F = 4.807$ ,  $df = 2, 15$ ,  $P = 0.024$ ). Tofu was used to preserve moisture content of pelts. Tukey's HSD comparisons and associated probability values are indicated by horizontal lines. (B) Profiles for the cumulative mean ( $\pm$ SEM) bait take of DNM,  $P_{nt}$ , and  $P_{alc}$  baits as a function of time ( $F = 4.643$ ,  $df = 2, 15$ ,  $P = 0.027$ ). Simple orthogonal planned contrasts and their associated probabilities are indicated by vertical lines. Percentage estimates were calculated using transect as the experimental unit ( $N = 6$ ) with 10 observations per bait type per transect.

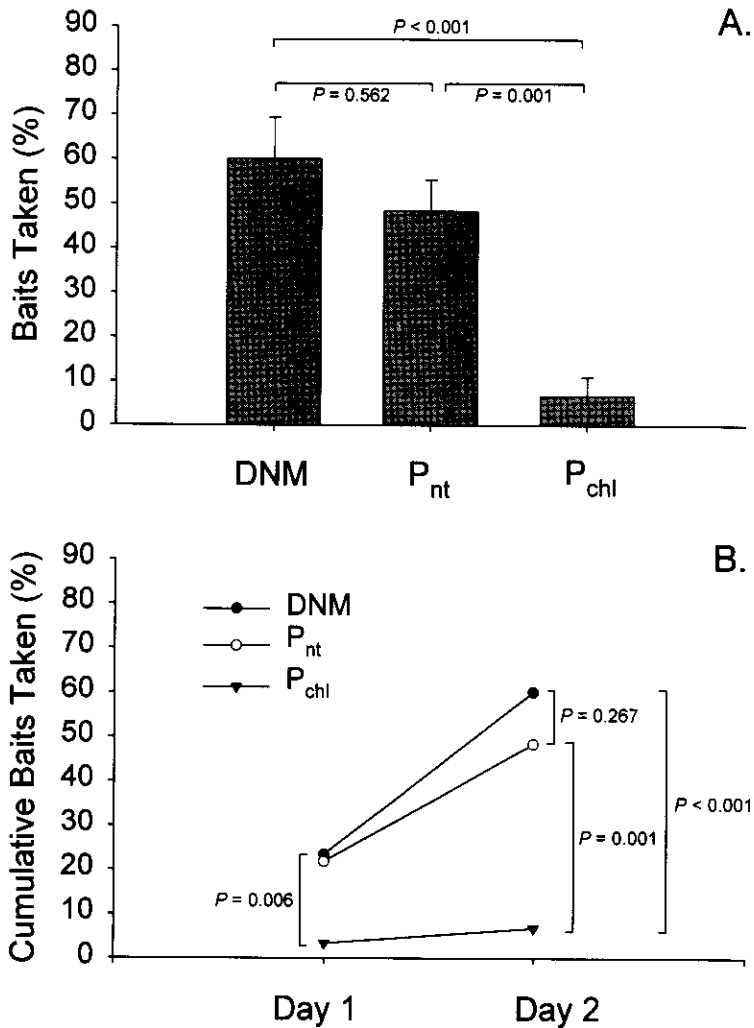


FIG. 6. (A) Bait take (mean  $\pm$  SEM) by brown treesnakes on Guam in experiment 6 for whole dead neonatal mice (DNM), nontreated mouse pelts wrapped around tofu (P<sub>nt</sub>), and bleach-treated mouse pelts wrapped around tofu (P<sub>chl</sub>) ( $F = 18.727$ ,  $df = 2, 15$ ,  $P < 0.001$ ). Tofu was used to preserve moisture content of pelts. Tukey's HSD comparisons and associated probability values are indicated by horizontal lines. (B) Profiles for the cumulative mean ( $\pm$ SEM) bait take of DNM, P<sub>nt</sub>, and P<sub>chl</sub> baits as a function of time ( $F = 4.877$ ,  $df = 2, 15$ ,  $P = 0.023$ ). Simple orthogonal planned contrasts and their associated probabilities are indicated by vertical lines. Percentage estimates were calculated using transect as the experimental unit ( $N = 6$ ) with 10 observations per bait type per transect.

surface decreases the attractiveness of the bait, either by chemical extraction of critical volatiles or by the antimicrobial effects of the alcohol wash.

*Experiment 6: Attractiveness of Baits Treated with Bleach.* Treatment of pelts with bleach strongly decreased the attractiveness of the bait (Figure 6A). Pelts treated with bleach were taken at 11% of the rate of whole dead mice, and at 14% of the rate of nontreated pelts. This strong main treatment effect held across days (Figure 6B). The source of the strong interaction effect was owing to the proportional cumulative increase in bait take for the whole dead mouse and nontreated pelts in contrast to the lack of increase in bait take for the bleach treated pelts. These observations indicate that bleach treatment exerted a stronger negative effect on bait take than an alcohol wash and that this action either by chemical extraction or antimicrobial properties eliminated important cues for foraging brown treesnakes that originated from the surface of the baits.

Both alcohol and bleach treatments of the pelts decreased microbial growth (Table 1). However, treatment with alcohol apparently did not eliminate all colony forming units because there was noticeable bacterial growth on agar plates after 48 hr. Bleach was a more effective disinfectant because none of the cultures showed evidence of any colony forming units for up to six days, after which some cultures showed signs of fungal growth.

From the field, a sample of unconsumed baits left in PVC tubes after two to three days was taken back to the laboratory and placed onto nutrient agar plates and incubated for 24 hr. All samples of whole dead mice and non-chemically-treated pelts yielded substantial bacterial growth after 24 hr of incubation (Table 1). The alcohol- and bleach-treated baits did not yield appreciable microbial growth after incubation. Of course, the microbial contamination of baits that were consumed is

TABLE 1. POSITIVE CULTURES FOR BAIT TREATMENTS PREPARED FOR BROWN TREE SNAKES ON GUAM IN MAY AND JUNE 2000

Treatment	Laboratory assays <sup>a</sup>		Field recovery assays at 24 hr <sup>b</sup>
	24 hr	48 hr	
DNM	10	10	10
P <sub>nut</sub>	10	10	10
P <sub>alc</sub>	3 <sup>d</sup>	7	1 <sup>d</sup>
P <sub>chl</sub>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>
Control <sup>c</sup>	0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>

<sup>a</sup> The number of agar plates out of 10 that showed signs of microbial growth. Freshly prepared baits were placed on nutrient agar cultures and checked at 24- and 48-hr intervals.

<sup>b</sup> The number of agar plates out of 10 that showed signs of microbial growth for bait treatments recovered from the field after >72 hr and checked after 24 hr of incubation on a nutrient agar culture plate.

<sup>c</sup> The negative control consisted of nutrient agar plate.

<sup>d</sup> Within-column comparisons of treatments to DNM ( $P < 0.05$ , binomial test).

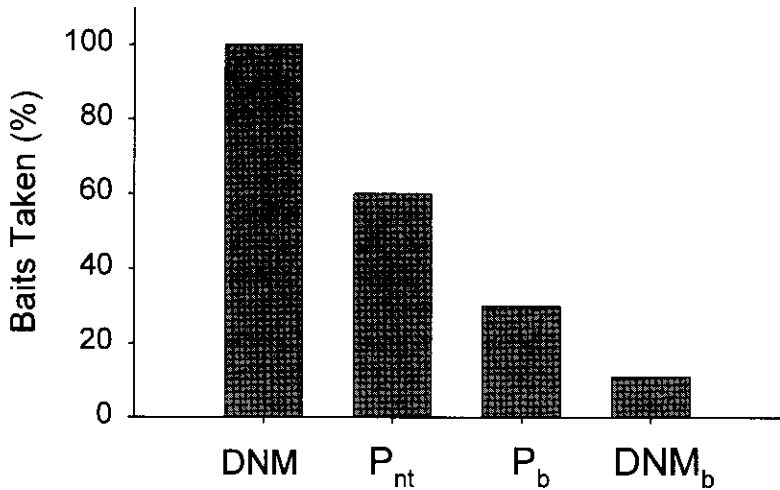


FIG. 7. Bait take by brown treesnakes on Guam for a single transect in experiment 7 for whole dead neonatal mice (DNM), nontreated mouse pelts wrapped around tofu ( $P_{nt}$ ), boiled mouse pelts wrapped around tofu ( $P_b$ ), and boiled whole mice ( $DNM_b$ ). Tofu was used to preserve moisture content of pelts. Estimates were based on 10 observations per treatment type for this single transect. All treatments ( $P_{nt}$ ,  $P_b$ ,  $DNM_b$ ) were different from DNM ( $P < 0.05$ , binomial test).

unknown. However, when the bait take rates of alcohol- and bleach-treated baits are considered in the context of the laboratory microbial culture experiments, the bait take rates are concordant with the degree of microbial contamination. That is to say, there is sufficient inferential evidence to suggest that the rate of bait take is dependent on the degree of microbial growth on the surface of the bait. Isolates from the cultures indicate that the predominant bacterial growth originating from the surface of dead mice is *Enterobacter agglomerans*.

*Experiment 7: Attractiveness of Denatured Baits.* Treating the mouse substrate by boiling also affects the attractiveness of baits (Figure 7), although there may also be effects owing to the general denaturation of the mouse parts.

## DISCUSSION

Future effective snake control programs on Guam will use both traps and toxic bait delivery. For traps, the current lure used is a live mouse contained within a cage inside the trap. Such traps are reasonably effective in depopulating modest areas of brown treesnakes (Engeman et al., 1998). One drawback with this method of control is the expense and effort of maintaining live mouse colonies in the lab

and maintaining the live mouse lures in the field (Clark et al., unpublished results). The use of toxic baits also can be effective at depopulating modest areas of forest of snakes. Savarie et al. (2001) employed whole dead neonatal mice laced with acetaminophen as the lure/bait-toxicant delivery system in achieving near zero survivorship of snakes in 6-ha experimental plots. Despite this success there still is a need to develop a lure/bait system that does not rely on the killing of mice.

The successful artificial lure/bait system must provide snakes with long-distance cues that act to attract the snake to the vicinity of the substrate and short-distance cues that will promote ingestion. These cues are not necessarily the same (Clark and Kimball, unpublished results). The difficulty in constructing artificial lure and bait systems has been the uncertainty of what these cues might be. Presentation of simple chemical components of mouse carrion have yielded variable, but generally poor, success as attractants (Shivik and Clark, 1999a; Clark and Kimball, unpublished results). Even reconstruction of mouse carrion odor based on chemical analysis of the volatiles produced during rotting have failed to successfully attract snakes (Shivik, 1998). Clearly, brown treesnakes, despite their anecdotal reputation for eating anything, are quite discriminating foragers. One possible solution in the development of a realistic mimic of complex carrion odor is to develop a system that includes the odor-producing agents and the appropriate nutrient substrate for those agents. This study is a critical first step in that process.

Throughout the experiments we found a concordance between factors relating to microbial decomposition of the mouse and the rate at which baits were taken by snakes: baits left in the field for two days were taken at higher rates, and chemically treated (alcohol or bleach) baits were taken at lower rates. These chemicals act as disinfectants, but they may also act as extractive solvents, removing critical volatiles. While we infer that the critical attractive volatiles are products of microbial metabolism, and our experimental evidence is consistent with this view, these chemicals may also have removed critical volatiles by extraction. Experiments using antibiotics to suppress bacterial growth would be useful in determining which hypothesis is responsible for the taking of baits by snakes. It is unlikely that chemical treatment resulted in residues that acted as repellents to snakes. The positive controls, DNM, were in PVC tubes that were cleansed with bleach, and taking of bait was consistently higher in those treatments.

We also found that the nature of the bait substrate was important. Mouse carcasses without skin and reconstituted ground-up mice were not good baits. Whole mice and pelts were good baits. These observations make sense in the context of the cues a foraging brown treesnake is likely to encounter. Typically, the initial odors generated from carrion will be the result of aerobic decomposition of the surface of the animal. Thus, snakes should be more attentive to these types of cues.

Future development of artificial lure/bait systems may include the cell culture of mouse skin as the nutrient substrate that could be embedded into agar blocks

and inoculated with appropriate bacteria. We suggest *Enterobacter agglomerans* as an initial odor-producing agent, because it is the dominant bacterium isolated from mouse skin, although other bacteria could contribute to the complex odor of carrion. These bioreactor systems could potentially act both as lure and bait systems. Such systems would greatly reduce the need for large numbers of live and dead mice for snake control, thus making progress toward the goal of using artificial lure/bait systems to achieve effective snake control. Future work in our laboratory is directed at addressing these issues.

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