DISSERTATION

MICROGEOGRAPHIC AND ONTOGENETIC VARIABILITY IN THE ECOLOGY OF
INVASIVE BROWN TREESNAKES ON GUAM, AND EFFECTS OF ROADS ON
THEIR LANDSCAPE-SCALE MOVEMENTS

Submitted by

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ABSTRACT

MICROGEOGRAPHIC AND ONTOGENETIC VARIABILITY IN THE ECOLOGY OF

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THEIR LANDSCAPE-SCALE MOVEMENTS

The accidental transportation of Brown Treesnakes (*Boiga irregularis*) to the remote Pacific island of Guam has resulted in a cascade of ecological changes starting with the loss of an entire forest avifauna due to hyperpredation by this adaptable generalist predator. These impacts – as well as the feeding ecology, population characteristics, and reproductive biology of the invader – were first documented in the 1980s by Dr. Julie A. Savidge. Since that time, there have been nearly a thousand publications with keywords of "*Boiga irregularis*," including over 350 journal articles (Gordon H. Rodda, unpublished literature survey). In addition to this scientific interest and public notoriety, the Brown Treesnake problem on Guam has elicited a comprehensive and costly management response to prevent the spread of snakes to other snake-free environs and to suppress populations on Guam with the ultimate goal of recovering native wildlife.

Since Savidge's early work, much Brown Treesnake science has focused on the basic biology and behavior of the snakes and efficacy of control technologies. Landscape-scale suppression is a primary management objective, but little research has been done on landscape-scale variation in snake population characteristics and effects of local ecological conditions on prospects for management success. I endeavored to provide managers with baseline data on the starting conditions for broad-scale management of this intractable invader while contributing to the body of scientific knowledge on the effects of landscape features on animal movement, microgeographic and ecological variation in population characteristics, interactions among native and non-native predators and prey, and changes in resource use throughout the ontogeny of organisms with indeterminate body size.

These objectives were met through two major projects: "Island-wide Visual Surveys" (Chapters One through Three) and "Roads as Barriers to Movement" (Chapters Four and Five).

Island-Wide Visual Surveys included the most comprehensive and controlled sampling of Brown Treesnakes – or, to our knowledge, any snake species – in the published literature. Eighteen sites, stratified by six habitat types, were selected across the entire geographic expanse of Guam, and a sample of 100 snakes was collected at each site. Together, these habitat types are representative of 95% of the terrestrial habitats on the island. Data on search efforts, capture locations, morphometrics, and necropsies were collected and analyzed to comprise the first three chapters of this dissertation.

Localized ecological conditions have the potential to induce variation in population characteristics such as size distributions and body conditions. The ability to generalize the influence of ecological characteristics on such population traits may be particularly meaningful when those traits influence prospects for successful management interventions. In Chapter One, I characterized the variability in invasive Brown Treesnake population attributes within and among habitat types. Quantile regression on snake size and body condition indicated significant ecological heterogeneity, with a general trend of relative consistency of size classes and body conditions within and among scrub forest and *Leucaena* forest habitat types and more heterogeneity among ravine forest, savanna, and urban residential sites. Larger and more robust snakes were found within some savanna and urban habitat replicates, likely due to the relative availability of larger prey. Compared to more homogeneous samples in the wet season, there was more variability in size distributions and body conditions in the dry season. Although there is evidence of ecological influence on Brown Treesnake populations at localized scales (e.g., the higher prevalence of larger snakes – particularly males – in savanna and urban sites), the level of variability among sites within habitat types indicated little ability to make meaningful predictions about these traits at unsampled locations. Seasonal variability within sites and habitats indicated that localized population characterization should include

sampling in both wet and dry seasons. Extreme values at single replicates occasionally influenced overall habitat patterns, while pooling replicates masked variability among sites. A full understanding of population characteristics should include an assessment of variability both at the site and habitat level.

Size structure within populations of invasive species may have consequences for relative risk at all stages of the invasion process, with obvious implications for management interventions such as interdiction, suppression, and eradication. In Chapter Two, I assessed relative distributions of invasive Brown Treesnakes among demographic categories of management interest. Samples indicated significant differences in distributions of female and male snakes among management classes (juvenile, transitional, and mature) by site and habitat. I found substantial heterogeneity in localized population characteristics over relatively small geographic distances, only modest influences of habitat type, higher prevalence of reproductively mature snakes in savanna and urban habitats, and a high proportion of snakes that are too small to be effectively targeted by current rodent-based control tools (mean = 38.2%, range = 19 to 72%). Failure to account for such variability in demographic fractions of elevated management risk may hinder successful interventions.

In Chapter Three, I examined the stomach contents of Brown Treesnakes to investigate microgeographic, ecological, seasonal, and ontogenetic variability of their diets throughout the island. We recovered 547 identifiable prey items from 1,789 snakes. Compared to historical and native range samples, current Brown Treesnake diets on Guam were characterized by a much lower proportion of bird and mammal prey and a high proportion of small lizards, particularly within forest habitats. Diet composition showed significant variability within and among habitat types and only minor seasonal variation. Diets in forest sites were almost exclusively comprised of small lizards (geckos and skinks), while savanna samples included more mammal prey and urban samples included more non-native birds and lizards. Proportions of snakes containing prey in stomach contents were strongly correlated with snake size and body condition. Diet composition exhibited little to no demonstrable variations between

wet and dry seasons. Diets varied considerably by size class of snake, with an ontogenetic shift from ectothermic prey (small lizards) to endothermic prey (birds and mammals) and the smallest prey being dropped from diets as snakes increased in size. Spatial heterogeneity in the distributions of prey – primarily the loss of birds and mammals from forest habitats resulting from predation by Brown Treesnakes – appears to drive size-dependent shifts in foraging activity, with larger snakes shifting to a more terrestrial foraging strategy and the largest snakes being found in non-forest habitats. Our sample is the first to document recently-introduced frogs as an appreciable portion of snake diets on Guam. Although the majority of individual prey items were of native or prehistorically introduced small lizard species, the majority of species preyed upon by Brown Treesnakes were non-native, reflective of the highly-invaded nature of Guam's habitats.

There is a rapidly growing body of evidence on the lethal and sub-lethal effects of roads on individuals and populations of many wildlife taxa. Because roads are the most ubiquitous landscape features on Guam and may be used as *de facto* boundaries for management units during future landscape-scale population suppression or eradication attempts, it is important to assess the potential impacts of roads and other habitat gaps (e.g., utility corridors) on the movements of snakes between fragments of forest, the preferred habitat type of Brown Treesnakes.

While a barrier effect of roads can have significant impacts on the dispersal of wildlife, posing a threat to the abundance and diversity of desirable flora and fauna, it can also afford some opportunity for enhancing control of invasive species. During the pilot phase of the "Roads as Barriers to Movement" project (Chapters Four and Five), I radio-tracked 45 free-ranging Brown Treesnakes in close proximity to two roads in Guam and recorded instances where daily relocations of snakes spanned roads. I then reconstructed observed movement histories with randomized turning angles, which served as a useful null hypothesis for assessing the effect of roads or road edge habitat on Brown Treesnake movement patterns. Through these "random walk" simulations I demonstrated that Brown Treesnakes

crossed these roads at a rate far lower than would be expected if snake movement was random with respect to roads and road edge habitat. I discussed two alternative hypotheses for these results: 1) habitat gaps posed by roads physically and/or behaviorally restrict snake movement, or 2) road edges provide preferred foraging habitat from which snakes are reluctant to depart. Because roads often form the boundaries of jurisdictional and management units, the effects of roads on the movement of invasive Brown Treesnakes will influence the prospects for success of future landscape-level suppression efforts.

In Chapter Five, I employed the same radio-tracking methods at an additional 48 sites across the expanse of the island, representative of the majority of the range of road, path, and utility corridor characteristics existing on Guam. I investigated the relative influences of road characteristics, context, environmental conditions, and individual traits of snakes on the probability of road crossings through short-term radio-tracking of 691 snakes within close proximity to gap segments. All measures of gap magnitude (e.g., gap width, surface type) were significantly negatively correlated with crossing probabilities, with traffic volume being the single most influential road characteristic. Body size was the only intrinsic factor associated with crossing rates, with larger snakes crossing roads more frequently. Despite plausible hypotheses about the effects of moonlight and precipitation, humidity was the only environmental variable affecting crossing rate. There was no difference in crossing rates between wet and dry seasons. The distance of the snake from the road at the start of nightly movement trials was the single most significant predictor of crossings. The presence of snake traps with live mouse attractants during a portion of the trials was associated with a decrease in crossing rates, indicating that strong localized prey cues influenced a snake's decision to cross. My results indicate that per capita road crossing rates of Brown Treesnakes are very low, and comparisons to historical records suggest that crossing rates have declined in the 60+ years since introduction to Guam.

striven to elevate myself to.

At the heart of it all is my mother, Ardell Ferguson, who always gave me the freedom and encouragement to pursue my interests and gave whatever emotional support I needed. Maybe most importantly, she got me hooked on books at a very young age. I'd like to thank my stepfather Frank Ferguson for his efforts to get me to occasionally pull my nose out of those books and pay attention to the rest of the world. My grandparents Lila and Gordon Ellestad taught me what it means to be truly good people. And my sister, Vicki Berberich, and her husband Kyle, have allowed me to vicariously lead a family life by occasionally peeking in and observing the growth of three fantastic nephews, Todd, Lane, and Brady. I also wish to acknowledge the father I never got to know, Robert Duane Siers; I often wonder how my life would have been different if he had lived to witness and guide its path.

Chapter One: Microgeographic Variation in Population Characteristics of an Invasive Vertebrate Predator

Introduction

One of the key unanswered questions in ecology is how the spatial structure of populations is influenced by ecological and demographic processes (May, 1999; Sutherland et al., 2013). Variability in resource requirements and the distribution of those resources may result in the uneven distribution of demographic fractions across a heterogeneous landscape. Most studies on the spatial structure of populations occur across large-scale ecological gradients (e.g., latitude or altitude) and seek to identify broad patterns in behavior, ecology or evolution. Sampling of a small number of populations at great distances and assuming monotonic changes between those populations may mask significant heterogeneity at finer spatial scales and make patterns appear more predictable at broader scales because effects of local heterogeneity are averaged out (Wiens, 1989). Averaging population characteristics over large areas can mask important localized population characteristics and dynamics such as variation in size distributions, reproductive habits, habitat affiliations, etc. Even seemingly homogeneous habitats may harbor structured subpopulations of vagile generalists (Ehrlich et al., 1975; Madsen and Shine, 1998), and population studies conducted at single sites may provide extremely biased conclusions (Kephart, 1982).

Within populations, different demographic segments (e.g., sexes, age classes) may exhibit dissimilar ecological relationships, and the nature of those differences may have consequences for population studies including those involving climate change, population and habitat viability analyses, etc. With respect to invasive species, smaller and more numerous age classes may more likely be accidentally transported, while mature individuals pose greater risk of reproduction upon relocation and continued recruitment during eradication efforts. Accounting for inter-site variability and habitat influence on relative proportions of individuals in those demographic strata may have consequences for success of proposed management

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interventions.

Sixty years after the introduction of Brown Treesnakes (*Boiga irregularis*), the island of Guam offers the opportunity to investigate the spatial variability of an invasive predator within and among a diversity of habitat types on a relatively small geographic scale. During growth, Brown Treesnakes undergo a pronounced ontogenetic shift from a diet consisting almost exclusively of ectothermic prey (small lizards) to preferred prey of endotherms (birds and mammals) as adults (Savidge, 1988; Greene, 1989; Lardner et al., 2009). Because current control technologies rely on rodents as trap or toxicant baits, the strong preference of juvenile snakes for small lizard prey renders these tools largely ineffective against snakes in smaller size classes (Tyrrell et al., 2009; Lardner et al., 2013). For this reason, along with a snake's transition into maturity as it grows (Savidge et al., 2007), snake body size is the individual characteristic of greatest importance with respect to its ecology and management. Intraspecific differences in snake body size may result in ontogenetic shifts in habitat use (Werner and Gilliam, 1984) as they target size-appropriate prey in a heterogeneous landscape. Along with body size, body condition can influence a snake's reproductive ability (Naulleau and Bonnet, 1996) and susceptibility to control tools (e.g., Tyrrell et al., 2009; Lardner et al., 2013), and therefore is also germane to variability within and among Guam's diverse habitats.

Since the 1980s and 1990s (e.g., Fritts and Scott, 1985; Savidge, 1988, 1991; Savidge et al., 2007), little effort has been dedicated to understanding the landscape-level ecology of the invasive population of Brown Treesnakes on Guam. Samples from prior demographic studies of Brown Treesnakes have either been based on small numbers of sites (Trembath and Fearn, 2008), pooled by large ecoregions (Greene, 1989; Shine, 1991a), or classified by broad habitat type (forest vs. non-forest; Savidge, 1991). These samples were obtained from museum collections (Greene, 1989; Shine, 1991a), road-cruising for live and road-killed snakes (Trembath and Fearn, 2008) or by combined visual searching, trapping, collection of road-kills, and donation by the public (Savidge, 1991), all of which involve inconsistent sampling

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biases which make precise, direct comparisons among samples difficult. Although objectives for the management of this damaging invasive species include landscape-scale suppression, little is currently known about how variation in Brown Treesnake population characteristics affects prospects for management success across Guam's heterogeneous landscape.

Here we report on one of the most comprehensive and standardized assessments of the geographic variability of any snake population, with the intent of characterizing the extent to which Brown Treesnake size distributions and body conditions vary throughout the landscape of Guam. Given the relative importance of snake body size and condition, and the potential for population-level variability in these traits among Guam's diverse habitats, we endeavored to answer the following research questions: 1) Do Brown Treesnakes demonstrate divergence in habitat use by sex and size classes? 2) Does body condition vary by habitat and sex? 3) Are there seasonal effects on body condition and habitat use by sex and size classes?

Methods

Study sites – The island of Guam is situated in the Philippine Sea roughly between New Guinea and Japan (13.2 to 13.7⁰ N and 144.6 to 145.0⁰ E). Although relatively small ($\sim$540 km^2), Guam is topographically and ecologically diverse, ranging from a wet northern limestone plateau historically dominated by moist, broadleaved evergreen forest, to a drier southern region with rolling hills and mountains of volcanic origin, largely comprised of savanna vegetation. Guam's climate is characterized by a warmer wet season running from July through November and a cooler dry season from December through June. While forest habitats remain green year-round, southern savannas and sites dominated by exotic *Leucaena leucocephala* ("Tangantangan") may become relatively arid and fire-prone during the dry season.

I selected three study sites within each of six habitat types as classified by Liu and Fischer (2005) following the nomenclature of Mueller-Dombois and Fosberg (1998). Sites were dispersed across the majority of Guam's geographic extent and ground-truthed to contain

large uninterrupted tracts representative of the respective habitat type (Figure 1.1).
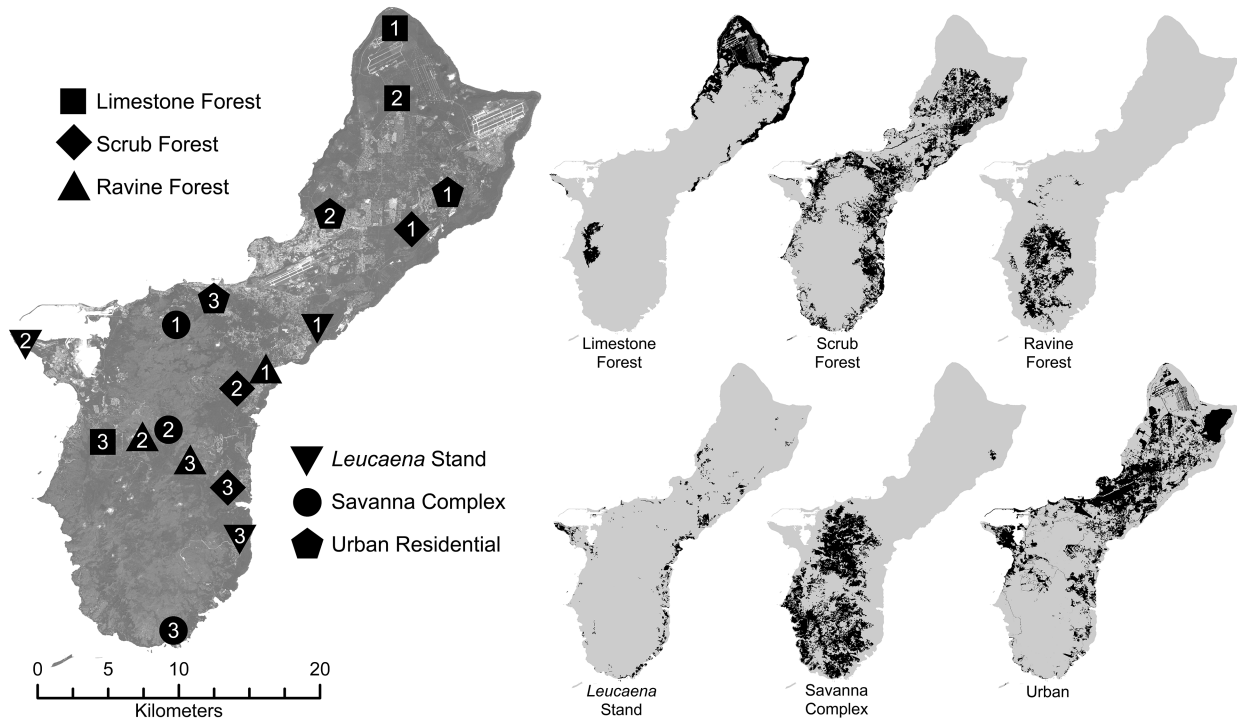
Figure 1.1: Habitat classification map of Guam depicting the distribution of the six target habitat types and the 18 sampling locations (after the classification of Liu and Fischer, 2005).

Habitat types – Limestone forest (LIM), 13% of Guam's land cover, is characterized by moist, broadleaved evergreen forest of predominantly native species on elevated limestone plateaus. Scrub forest (SCR) is variable and composed of primarily non-native species resulting from a long history of human disturbance. It covers 23% of Guam's land mass and comprises 58% of total forest cover. Ravine forests (RAV), 8% of Guam's habitat, are low-lying areas surrounding flowing and ephemeral watercourses, and are primarily moist green forests containing higher proportions of palms, bamboos, and *Pandanus* sp. *Leucaena leucocephala* (LEU) is an introduced species providing excellent habitat for Brown Treesnakes. Nearly all forested areas on Guam have some amount of *Leucaena*, particularly at forest edges; however, in some areas it forms nearly monotypic stands that cover 3% of Guam's land area. Savanna complex (SAV) is a mosaic dominated by grasses with emergent shrubby vegetation and erosion scars, and comprises a significant proportion of Guam's southern

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region (21% of Guam's total area). Urban built-up and landscaped areas, 27% of Guam's habitats, include industrial, commercial and residential areas. For reasons of consistency, access, and public awareness, we elected to concentrate our surveys in and around urban residential (URB) areas. Together, these six habitat types comprise 95% of Guam's land cover.

Survey methods – My field team employed visual survey methods to sample snake populations at the eighteen selected sites. Visual surveys provide low yield per unit effort when compared to trapping, but samples are more representative of the population and exhibit less size bias (Rodda et al., 2007). Surveys commenced at sunset and were conducted for three to four hours, which includes much of the peak activity period of Brown Treesnakes (Rodda et al., 1999; Lardner et al., 2014). These trained searchers, equipped with powerful headlamps, followed habitat edges at roughly 0.5 km per hour, examining all visible vegetation and non-vegetative structure for the presence of snakes. Forest habitats were surveyed from road or trail edges. Savanna searches included road edges, footpaths, and trackless searches throughout the habitat mosaic, including edges of erosion scars. Urban searches were conducted by searching residential yards, examining all structures and vegetation for the presence of snakes; yards were separated from large forest tracts by at least one paved road, as Brown Treesnakes tend to avoid crossing roads (Siers et al., 2014). Searchers stopped searching when encountering habitat formations inconsistent with the search objectives and resumed searching upon returning to representative habitat.

Sampling objectives – To obtain enough data to accurately describe size distributions, we collected a target sample of 100 snakes from each site, for a total of approximately 1,800 snakes. To minimize the potential for bias resulting from short-term population dynamics or seasonal effects, sample sizes were balanced between wet and dry seasons, and each season was sampled in at least two quarterly bouts. The one exception was the second limestone forest replicate (LIM2), which was sampled in one relatively continuous effort due to impending construction of a snake-proof barrier; in this case, 90 snakes were collected in the wet season

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and 10 in the dry season.

Snake processing – Upon visual detection, an attempt was made to hand-capture the observed snake. Following capture, searchers recorded time and location of capture, microhabitat characteristics, and morphometric data. Morphometric data include snout-vent length (SVL), obtained by stretching the snake along a flexible tape ruler, and weight using Pesola spring scales (Pesola AG, Baar, Switzerland). Captured snakes were transported to the U.S. Geological Survey Brown Treesnake Lab the following morning, where SVL and weight were re-measured to validate field measurements. Snakes were euthanized and necropsied. Sex was determined by examining internal reproductive physiology. All animal use was conducted in accordance with Colorado State University IACUC Protocol #09-1436A.

Body condition – I assessed linear, quadratic, third-, and fourth-order polynomial models for fit to a regression of the log of snake body mass (subtracting the mass of any stomach contents) against the log of snake SVL for all sampled snakes, with model selection performed by comparing Akaike Information Criteria (AIC) values. I scaled residuals from the top model so that values are in units of standard deviations from the predicted value. A snake with a body condition (CI) value of -0.5, then, was one-half of one standard deviation below the average body condition, and 95% of all snakes had CI scores $< +1.96$ and > -1.96, with a mean of zero.

Quantile regression – While most snake population studies report mean, median or modal SVL values and standard deviations to describe variation in body size (e.g., Parker and Plummer, 1987), these measures can be poor descriptors of potentially complex size distributions and may mask meaningful features of those distributions. Quantile regression allows estimation and inference on quantiles of a distribution without assumptions of normality and equality of variance required for standard regressions on mean values (Koenker and Bassett, 1978; Cade and Noon, 2003), and has previously been used to model geographic variation in growth and body condition for fish populations (Cade et al., 2008, 2011). Because different quantiles of Brown Treesnake body size may vary in meaningful ways with respect

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to interdiction and control (i.e., smaller quantiles are refractory to most control methods and larger quantiles constitute greater threats of reproduction), I determined that quantile regression was an appropriate approach to estimating the effects of habitat type, sex, and seasonal variation throughout the range of snake body length quantiles. I followed the same procedures to assess inter-quantile differences in body condition.

I generated quantile regression fits for every 2.5^{th} percentile from the 0.05^{th} to 0.95^{th} quantiles of SVL and CI values for each sex at each site using the following model:

$$Q(\tau_{0.05-0.95}|X) = \beta_{int} + \beta_{sex} + \beta_{site} + \beta_{sex*site} \tag{1}$$

where Q was the estimate of the τth quantile given the data (X), and β_{int} was the regression intercept term. I coded model contrasts such that quantile means were an average of all sites by sex, so estimates for female quantiles were compared to the average of all females at all other sites, while male estimates were compared to mean male estimates. I obtained quantile estimates and standard errors (SE) with the linear quantile regression function 'rq()' from 'quantreg' package version 5.05 (Koenker et al., 2013) for R version 3.0.2 (R Core Team, 2013), and estimated 95% confidence intervals at $Q \pm 1.96*$SE. To investigate habitat and seasonal effects on quantile estimates, I pooled replicates by habitat type and added a season covariate along with $sex * season$ and $sex * habitat$ interaction terms:

$$Q(\tau_{0.05-0.95}|X) = \beta_{int} + \beta_{sex} + \beta_{hab} + \beta_{seas} + \beta_{sex*hab} + \beta_{sex*seas} + \beta_{hab*seas} \tag{2}$$

where hab is a categorical covariate for each of the respective habitat types and $seas$ indicates wet or dry season. Contrasts were coded so that sex-specific estimates were compared to the respective means averaged over all habitat types and both seasons.

Results

At each of the 18 study sites, we captured a mean of 100 snakes (n = 99 to 104) for a total of 1,804 snakes (990 males and 814 females). Summary statistics on sample size, snake length, mass, and body condition are reported by sex in Tables 1.1 through 1.3, with these metrics further summarized by pooling them into forest (limestone, scrub, ravine, and *Leucaena*) and non-forest (savanna and urban) habitat types in Table 1.4. The largest snake captured was a 1950-mm male weighing 2,478 g, and the smallest a 350-mm, 7-g hatchling male. Quantile regression of sex-specific size distributions for the entire sample (i.e., with all other covariates at their mean values) revealed no significant differences in mean SVLs of sexes in all quantiles up until about the 0.85th quantile. At around 1000 mm SVL quantile means of male snakes became drastically and significantly higher than those of females (Figure 1.2A). Quantile estimates of sex-specific body conditions (CI) showed a relatively normal cumulative distribution for both sexes, but with the female estimate significantly higher along the entire quantile range (Figure 1.2B).
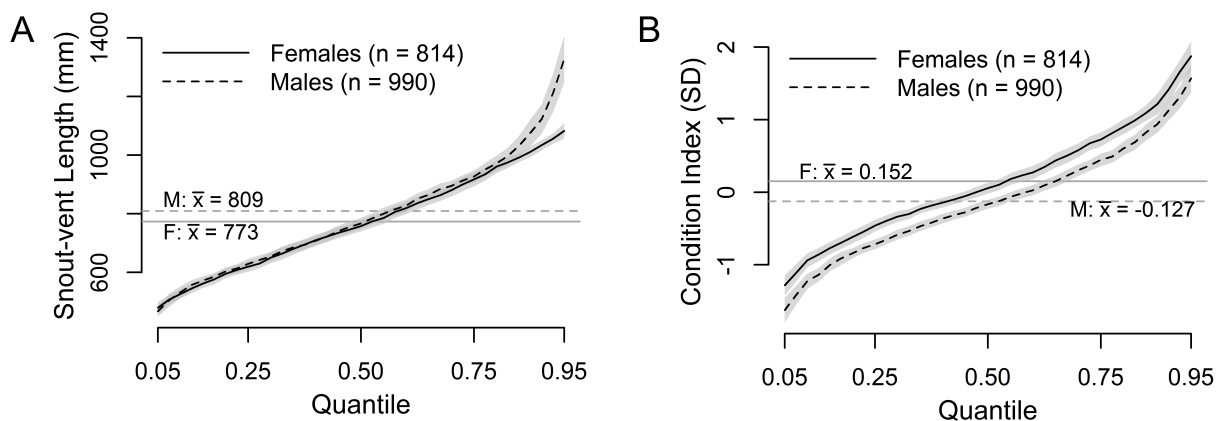
Figure 1.2: Quantile regression intercept values (cumulative distribution functions) comparing male and female body size (A) and condition index (B). Lines connect fit estimates for each quantile, and polygons represent the 95% confidence intervals for the estimates. Non-overlapping confidence polygons indicate significant differences.

Interactions of sex and site (per Equation 1), grouped by habitat type, indicate the level of variability among replicates within and among habitat types in SVL (Figure 1.3). Size

Table 1.1: Summary of snout-vent length (mm) by habitat type, replicate, and sex. 25% and 75% = 25th and 75th percentiles, or first and third quartiles. "LIM" = limestone forest; "SCR" = scrub forest; "RAV" = ravine forest; "LEU" = *Leucaena* stand; "SAV" = savanna complex; "URB" = urban residential.

Hab	Sex	Replicate 1						Replicate 2						Replicate 3						Habitat Pooled					
		N	Min	25%	Med	75%	Max	N	Min	25%	Med	75%	Max	N	Min	25%	Med	75%	Max	N	Min	25%	Med	75%	Max
LIM	M	55	502	712	818	945	1305	55	409	623	790	989	1597	42	391	567	723	851	1186	152	391	629	789	942	1597
	F	45	550	796	878	985	1072	45	378	691	801	965	1128	59	427	634	754	910	1128	149	378	676	836	965	1245
SCR	M	53	400	555	760	884	1123	70	506	621	762	879	1950	57	417	592	666	856	1209	180	400	601	716	871	1950
	F	51	400	583	742	899	1063	30	408	613	714	961	1404	43	460	590	770	913	1064	124	400	604	743	916	1404
RAV	M	39	356	723	878	996	1920	45	418	579	698	786	1549	50	354	517	582	788	1947	134	354	564	718	891	1947
	F	61	424	748	872	983	1083	56	398	629	722	835	1221	49	382	533	657	762	1167	166	382	611	750	877	1221
LEU	M	68	454	635	754	928	1395	63	466	656	761	925	1210	69	565	689	782	902	1170	200	454	664	765	922	1395
	F	33	501	625	727	827	1057	36	517	636	808	932	1140	31	567	648	774	930	1080	100	501	632	767	900	1140
SAV	M	65	375	650	796	922	1295	53	388	703	903	1174	1563	53	350	654	816	1015	1876	171	350	660	827	998	1876
	F	35	370	554	602	724	1030	49	505	657	745	906	1143	48	393	554	661	885	1346	132	370	571	694	866	1346
URB	M	55	408	636	775	912	1565	54	402	652	804	1207	1687	46	501	683	794	954	1779	155	402	643	784	981	1779
	F	45	395	604	718	844	1355	46	486	754	934	1052	1475	54	382	639	759	894	1165	145	382	658	779	954	1475

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Table 1.2: Summary of snake mass (g) by habitat type, replicate, and sex. 25% and 75% = 25th and 75th percentiles, or first and third quartiles. "LIM" = limestone forest; "SCR" = scrub forest; "RAV" = ravine forest; "LEU" = *Leucaena* stand; "SAV" = savanna complex; "URB" = urban residential.

Hab	Sex	Replicate 1						Replicate 2						Replicate 3						Habitat Pooled					
		N	Min	25%	Med	75%	Max	N	Min	25%	Med	75%	Max	N	Min	25%	Med	75%	Max	N	Min	25%	Med	75%	Max
LIM	M	55	14	35	49	68	297	55	9	23	42	94	907	42	7	17	32	53	159	152	7	24	42	70	907
	F	45	19	44	60	89	131	45	8	30	48	91	235	59	8	22	34	68	148	149	8	28	51	81	235
SCR	M	53	8	16	40	64	132	70	14	24	37	63	1727	57	9	20	27	54	233	180	8	21	33	62	1727
	F	51	8	24	42	73	147	30	9	24	32	75	520	43	11	20	42	77	153	124	8	22	41	74	520
RAV	M	39	6	36	57	83	1714	45	6	18	27	37	483	50	5	14	18	40	997	134	5	17	30	59	1714
	F	61	9	39	63	93	161	56	6	19	31	46	244	49	7	15	22	36	199	166	6	21	36	63	244
LEU	M	68	10	23	36	74	329	63	15	29	41	67	214	69	18	31	44	65	161	200	10	28	41	70	329
	F	33	14	25	37	55	141	36	16	27	46	73	202	31	15	27	41	72	159	100	14	26	41	71	202
SAV	M	65	7	25	43	76	279	53	7	33	66	182	774	53	5	25	53	110	1435	171	5	26	53	102	1435
	F	35	6	16	20	37	108	49	12	26	37	79	271	48	7	19	31	73	375	132	6	19	31	64	375
URB	M	55	8	23	38	62	794	54	6	29	68	254	1240	46	12	25	41	87	1307	155	6	25	41	95	1307
	F	45	7	22	33	52	428	46	15	45	92	172	667	54	7	25	37	73	233	145	7	26	43	103	667

Table 1.3: Summary of snake body condition index (CI) by habitat type, replicate, and sex. 25% and 75% = 25th and 75th percentiles, or first and third quartiles. Units are standard deviations from the mean of standardized residuals from a length by mass regression. "LIM" = limestone forest; "SCR" = scrub forest; "RAV" = ravine forest; "LEU" = *Leucaena* stand; "SAV" = savanna complex; "URB" = urban residential.

Hab	Sex	Replicate 1						Replicate 2						Replicate 3						Habitat Pooled					
		N	Min	25%	Med	75%	Max	N	Min	25%	Med	75%	Max	N	Min	25%	Med	75%	Max	N	Min	25%	Med	75%	Max
LIM	M	55	-4.16	-1.01	-0.42	0.15	2.00	55	-1.60	-0.34	0.01	0.50	2.01	42	-2.12	-1.00	-0.46	-0.04	1.56	152	-4.16	-0.85	-0.21	0.31	2.01
	F	45	-3.65	-0.49	-0.18	0.17	1.23	45	-1.92	-0.35	0.03	0.43	1.80	59	-2.01	-0.85	-0.50	-0.12	0.98	149	-3.65	-0.68	-0.19	0.16	1.80
SCR	M	53	-1.99	-0.37	0.04	0.60	1.91	70	-1.37	-0.60	-0.21	0.41	1.67	57	-1.63	-0.57	-0.27	0.12	1.72	180	-1.99	-0.56	-0.15	0.36	1.91
	F	51	-2.24	-0.07	0.56	1.04	2.84	30	-4.62	-0.27	0.21	0.78	1.50	43	-1.43	-0.19	0.09	0.72	2.30	124	-4.62	-0.17	0.30	0.88	2.84
RAV	M	39	-2.65	-0.78	-0.26	0.32	3.35	45	-3.10	-1.36	-0.56	-0.26	0.64	50	-4.59	-1.17	-0.88	-0.45	0.83	134	-4.59	-1.17	-0.63	-0.17	3.35
	F	61	-1.57	-0.39	0.33	0.83	3.33	56	-2.20	-1.03	-0.34	-0.08	1.31	49	-4.22	-0.92	-0.62	-0.18	1.25	166	-4.22	-0.85	-0.31	0.30	3.33
LEU	M	68	-1.95	-0.65	-0.32	0.19	1.47	63	-1.63	-0.37	0.14	0.98	1.99	69	-2.65	-0.77	-0.15	0.33	1.72	200	-2.65	-0.65	-0.14	0.47	1.99
	F	33	-1.36	-0.06	0.47	0.84	2.54	36	-1.28	-0.06	0.27	0.73	1.66	31	-1.76	-0.32	0.27	0.73	1.10	100	-1.76	-0.12	0.32	0.75	2.54
SAV	M	65	-2.46	-0.48	-0.11	0.34	1.89	53	-2.70	-0.72	-0.25	0.17	2.28	53	-2.82	-0.17	0.41	1.11	2.28	171	-2.82	-0.52	-0.03	0.63	2.28
	F	35	-1.60	-0.45	-0.05	0.53	1.57	49	-1.79	-0.39	-0.11	0.53	3.49	48	-2.55	0.02	0.69	1.47	4.06	132	-2.55	-0.35	0.16	0.93	4.06
URB	M	55	-3.02	-0.82	-0.16	0.42	1.51	54	-2.33	0.27	0.78	1.75	3.72	46	-3.53	-0.76	0.06	0.69	2.33	155	-3.53	-0.55	0.25	0.81	3.72
	F	45	-2.04	-0.62	0.09	0.59	1.89	46	-1.25	0.89	1.66	2.23	3.73	54	-1.87	-0.52	0.06	0.83	3.61	145	-2.04	-0.31	0.49	1.44	3.73

Table 1.4: Summary of snake body length, mass, and condition index pooled by forest and non-forest habitat types and by sex.

Hab	Sex	N	Snout-vent Length (mm)							Mass (g)							Condition Index (sd)						
			Min	5%	25%	Med	75%	95%	Max	Min	5%	25%	Med	75%	95%	Max	Min	5%	25%	Med	75%	95%	Max
Forest	M	666	354	466	618	753	907	1169	1950	5	11	23	37	65	164	1727	-4.59	-1.63	-0.77	-0.27	0.28	1.26	3.35
	F	539	378	485	633	768	917	1057	1404	6	11	24	41	73	130	520	-4.62	-1.30	-0.53	-0.03	0.58	1.30	3.33
Non-forest	M	326	350	487	650	805	990	1479	1876	5	12	25	46	101	532	1435	-3.53	-1.44	-0.53	0.04	0.74	2.19	3.72
	F	277	370	482	603	743	913	1142	1475	6	13	22	37	79	247	667	-2.55	-1.24	-0.34	0.27	1.14	2.46	4.06

distributions for males were relatively consistent among sites within limestone, scrub, ravine, *Leucaena*, and savanna habitats. Females also showed relative consistency in scrub and *Leucaena* habitat but considerable variability, including extreme deviation of some replicates from quantile averages, was observed in the other habitats. More specific observations include, but are not limited to: particularly large males in the higher quantiles at all ravine sites and some savanna and urban sites (SAV2, URB3); overall low female size distributions at RAV3 and SAV1, a high mid-range of females at RAV1, and an overall large size distribution of females at URB2; and relatively large females in the smaller quantiles of LIM1 but average sizes in the larger quantiles.

Figure 1.3: Comparison of male and female SVL quantile regression fits for each of the 18 sampled sites (three replicates per habitat type) across the quantile range from 0.05 to 0.95 (x-axes). The horizontal line at y = 0 depicts the quantile average for the respective sex from all sampled sites. A segment of the confidence polygon that does not include the average line indicates a significant difference at the respective quantiles.

Interactions of sex and site (Equation 1) with CI as the response variable (Figure 1.4) showed males with relatively consistent and "average" CI in scrub and ravine forest sites and female CI more consistent in limestone, scrub, and *Leucaena* habitat. In most cases, the behavior of the quantile fits was relatively parallel to the average values with the exception of LIM1 males and SAV3 and URB2 females. RAV2 and RAV3 females were in particularly

low body condition, while URB2 females were in remarkably high body condition.

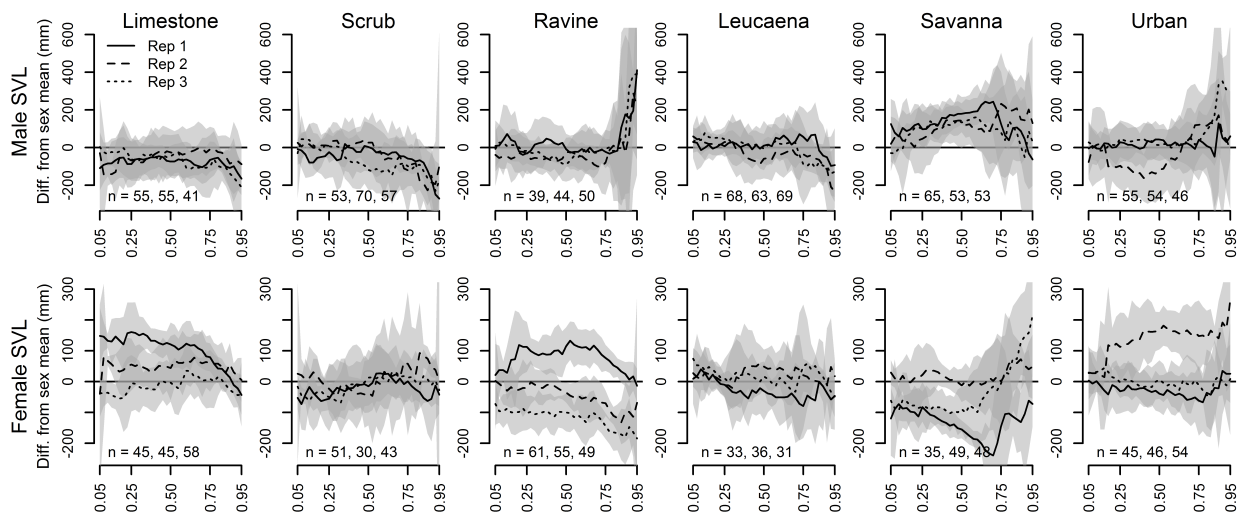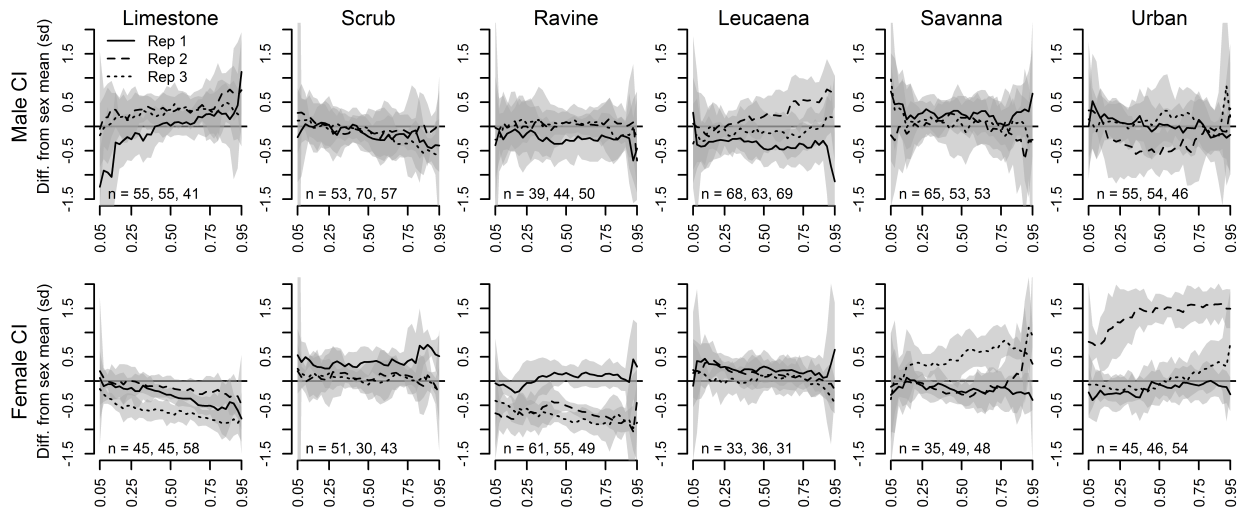
Figure 1.4: Comparison of male and female CI quantile regression fits for each of the 18 sampled sites (three replicates per habitat type) across the quantile range from 0.05 to 0.95 (x-axes). The horizontal line at y = 0 depicts the quantile average for the respective sex from all sampled sites. A segment of the confidence polygon that does not include the average line indicates a significant difference at the respective quantiles.

The results of the SVL and CI quantile regression models that pooled captures by habitat type (Equation 2) describe the size distributions by habitat type and the variation in quantile estimates among habitats (Figure 1.5). Males in limestone forest tended to be below average length along the quantile range, particularly at the larger quantiles, while savanna males tended to be significantly larger than average across the quantile range. Males had noticeably higher than average lengths in the highest quantiles among ravine forest, savanna and urban sites, and below average lengths in those same quantiles for limestone, scrub and *Leucaena* forest. The higher quantile estimates for female SVL showed the largest snakes in savanna and urban habitat, similar to (but not as distinctive as) the pattern in male snakes, and ravine forest females tended to be well below average in the higher quantiles. Limestone forest females tended to be larger across the mid-quantile range, but smaller than average in the higher quantiles.

CI results under the same model (Equation 2, Figure 1.5) indicated a striking divergence

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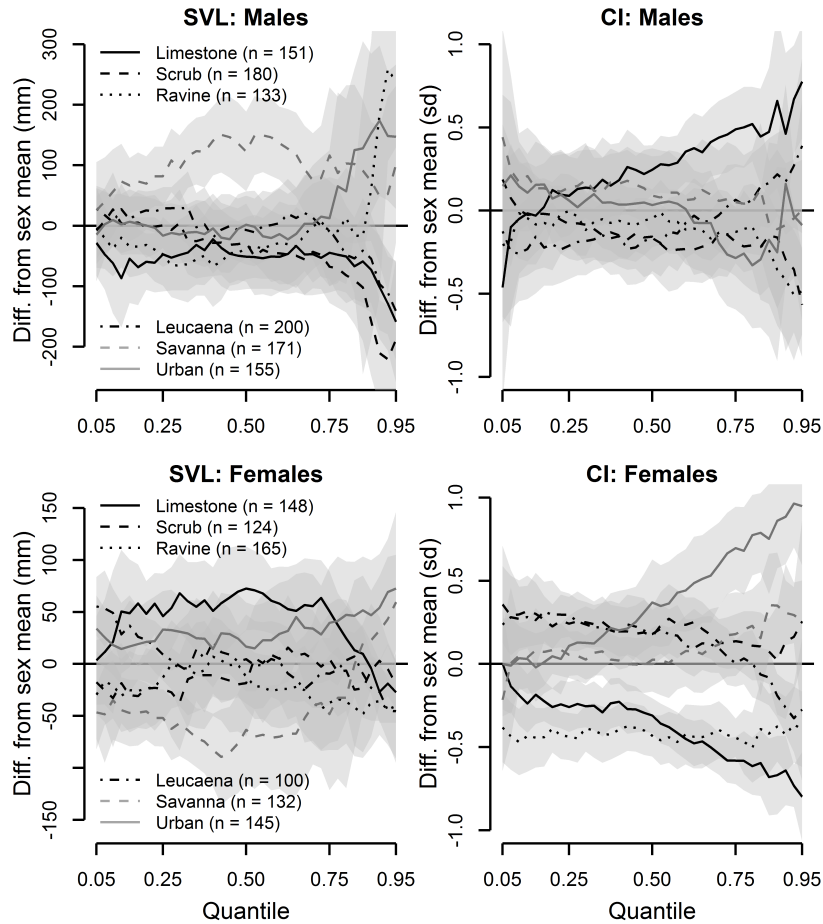
Figure 1.5: Quantile fits by habitat type for male and female Brown Treesnake SVL and CI, indicating the variability among habitat types. The horizontal line at y = 0 depicts the quantile average for the respective sex from all sampled sites. A segment of the confidence polygon that does not include the average line indicates a significant difference at the respective quantiles.

in the body conditions of male and female snakes in limestone forest, with very high-CI males and low-CI females, particularly in the higher quantiles. While ravine forest males tended to be relatively average in body condition, with the exception of the highest quantiles, ravine forest females were in consistently poor body condition across the entire quantile range. Although urban male snakes were of relatively average body condition, the highest body conditions were observed in the upper quantiles of urban female snakes, superficially similar but opposite to the pattern of body condition in limestone forest snakes.

Some of these deviations in the pooled habitat samples appear to be driven by more

extreme deviations within single replicates – e.g., the high SVL values in the upper quantiles of urban females result from the overall high female SVL values at URB2, while URB1 and URB3 are more average.

The habitat model (Equation 2) also included a seasonal term with sex-by-season and habitat-by-season interactions. For both SVL and CI, there was little effect of season as a whole for either sex, with the exception of marginally larger and more robust (higher-CI) male snakes found in the higher quantiles during the wet season than in the dry season (Figure 1.6).
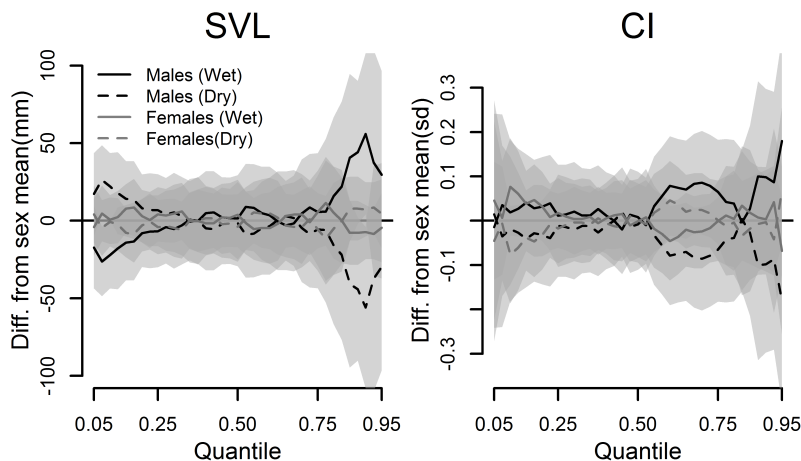
Figure 1.6: Seasonal differences in SVL and CI quantile estimates, averaged across all habitat types. The horizontal line at y = 0 depicts the quantile average for the respective sex from all sampled sites. A segment of the confidence polygon that does not include the average line indicates a significant difference at the respective quantiles.

There was very little influence of season on SVL or CI of female snakes in any portion of the quantile range. However, more seasonal influence on SVL and CI was evident when examining the season-by-habitat interactions. In general, SVL distributions tended to more closely reflect the quantile averages during the wet season, with the most dramatic deviations from average occurring in the dry season (Figure 1.7). Scrub forest males skewed particularly low in the higher quantiles during the dry season, while savanna males were consistently large and ravine forest males spiked in size at the highest quantiles. The upper quantiles of urban males appeared larger than average only during the dry season. Limestone forest females

tended to be relatively large across the mid-quantile range, and savanna females quite small, during the dry season.

Figure 1.7: Wet season and dry season SVL quantile estimate comparisons for both sexes by habitat type. The horizontal line at y = 0 depicts the quantile average for the respective sex from all sampled sites. A segment of the confidence polygon that does not include the average line indicates a significant difference at the respective quantiles.

A similar pattern of average distributions during the wet season and more extreme deviations during the dry season was evident in the CI habitat-by-season interaction estimates (Figure 1.8). In particular, a dry season spike in the higher CI quantiles was evident for limestone forest males and urban females, with below-average CIs for limestone and ravine forest females during the dry season.

Discussion

Do Brown Treesnakes demonstrate divergence in habitat use by sex and size classes? I observed the greatest proportion of very small male snakes in limestone forest habitat and the fewest in savanna habitat. Very small females, however, were more prevalent in savanna habitat while the lowest quantiles of females tended to be larger in *Leucaena* habitat. With respect to the quantile means, lower-quantile estimates were relatively average, particularly for small males, in the other habitats. Smaller snakes in the lower quantiles tended to
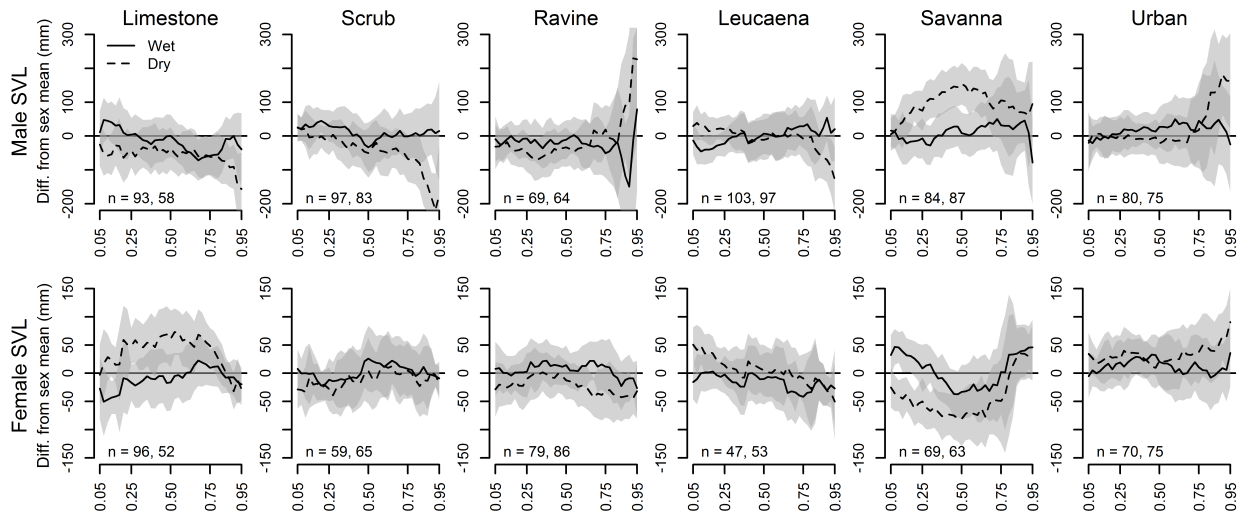
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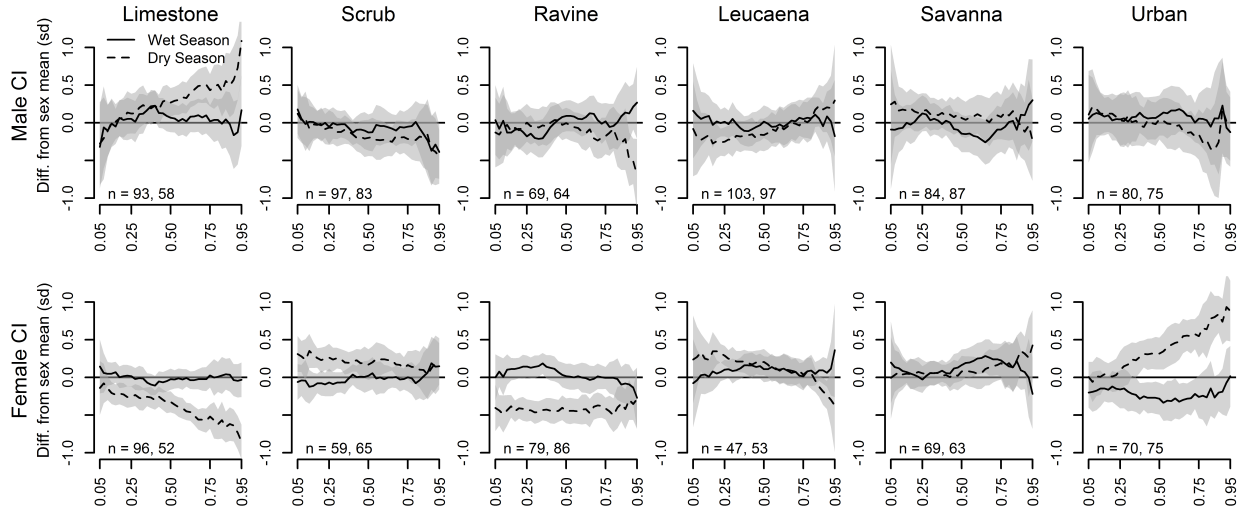
Figure 1.8: Wet season and dry season CI quantile estimate comparisons for both sexes by habitat type. The horizontal line at y = 0 depicts the quantile average for the respective sex from all sampled sites. A segment of the confidence polygon that does not include the average line indicates a significant difference at the respective quantiles.

be observed in outlier replicates, e.g., females from RAV3, SAV1, and SAV3 (Figure 1.3). Although sampling occurred over the course of one to two years in a seasonally-balanced fashion, it is possible that an excess of very small snakes at a few sites may be the result of conditions that led to high localized breeding and hatching rates. Throughout the median quantiles, snakes in most habitats were relatively invariant in size. The most extreme deviations in median size occurred at only one or two sites within habitat classes (e.g., LIM1, RAV1 and 3, SAV1 and 3, and URB2 females). Pooling by habitat, median sizes for female snakes in limestone forest habitats were significantly higher than average while males were about average or slightly smaller than average. Conversely, median male snake size was significantly higher than average in savanna habitats, while females were significantly smaller (Figure 1.7). While we can offer no intuitive process that might drive such differences in the limestone forest habitat, it is possible that the skew toward larger males and smaller females at savanna sites may in part be due to the greater role of rodents in the diets of Brown Treesnakes in this habitat (Savidge, 1988; Gragg et al., 2007; Siers, unpublished data). Geographic variation in sexual size dimorphism resulting from variation in prey availability

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has been observed in Australian pythons (Pearson et al., 2002a), and it is possible that this phenomenon can be observed among habitat types varying in available prey types; juvenile male Brown Treesnakes grow at a faster rate than females (Bjorn M. Lardner, unpublished data), and it is plausible that faster-growing male snakes may be more successful at bridging the gap in prey size between small lizards and large grassland rodents, leading to increased growth of males and suppressed growth of females. However, this might lead one to expect particularly low body conditions for savanna females, and this does not seem to be the case (1.5). Emigration of nutritionally stressed individuals from savanna habitat may account for the deficit of mid-sized females without an obvious body condition pattern, but this would likely be evidenced by a greater male bias in sex ratios in savannas compared to other habitats; no such pattern was apparent in our data (Chapter Two).

The most striking divergence in habitat use by size classes is that the highest quantiles of snakes are larger in savanna and urban habitats for both males and females, though there is much variability among replicates. This is likely the result of availability of larger prey such as rodents in savanna grasslands and human-commensal prey species (rodents, invasive birds, poultry) in urban residential areas (Savidge, 1988; Chapter Three). There is also a spike in male SVL at the highest quantiles in ravine forest. Ravine forests harbor abundant large frogs which may be preyed upon by larger snakes; however, observations of large frogs in the stomach contents of snakes is relatively rare (Chapter Three). While we captured more snakes of reproductive size in urban habitats, particularly URB2 for females, the lack of an excess of hatchlings in urban habitats (as would be indicated by lower estimates in the lower quantiles at urban sites) suggests that either young are not surviving, are more difficult to detect in this habitat, or oviposition is occurring in other habitats and residential areas may merely be transient feeding grounds. However, the presence of large females at SAV3 does coincide with the presence of smaller females in the lower quantiles.

Does body condition vary by habitat and sex? Throughout the entire quantile range of CI values, females consistently exhibited higher body condition (Figure 1.2B). As the metabolic

demands of reproduction fall more heavily on female Brown Treesnakes, they may invest growth capital in energy stores (e.g., Brown and Weatherhead, 1996; Madsen and Shine, 2002) and a more robust somatic mass rather than body length; Savidge (1991) observed a similar pattern among urban Brown Treesnakes on Guam and suggested this result supported the hypothesis that females are channeling growth resources into eggs or fat storage rather than length. There was generally more variability in body condition within savanna and urban habitats. Additionally, male snakes showed considerable variability among replicates in limestone and *Leucaena* forest while replicates for female snakes were variable in ravine forest. Female snakes demonstrated some of the most extreme highs (SAV3, URB2) and lows (LIM3, RAV2, RAV3) of body condition (Figure 1.4B). Differences in body condition between males and females within a single site were sometimes extreme: LIM1 males showed a very broad distribution of CI values (high values in the upper quantiles and low values in the low quantiles) while females showed a more narrow distribution (higher values in lower quantiles and lower values in higher quantiles) averaged below the mean; at URB2, CI values for females were remarkably high across all quantile ranges while male CI values were mostly low, with this distinction being even more pronounced when considering the female baseline was higher than the male baseline. Among pooled limestone forest sites, the general trend was for higher CI variance and mean among males and a lower variance and below-average CI among females. This pattern was nearly reversed for urban habitats, with female CIs much higher than male CIs in the higher quantiles (largely driven by extreme values at URB2). While male CIs in ravine forests were average (RAV2 and 3) to low-average (RAV1), female CIs in RAV2 and 3 were consistently low, indicating ravine forests, along with limestone forests, provide relatively poor opportunity for amassing metabolic stores for reproduction by females (Figure 1.5), consistent with low prey availability.

Are there seasonal effects on body condition and habitat use by sex and size classes? Season appeared to have little influence on the sizes of snakes detected during night searches, with the exception of a marginal increase in the size of male snakes in the higher quantiles

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during the wet season (Figure 1.6). As with body condition, the majority of variability in seasonal SVL distributions occurred in the dry season, while wet season samples were relatively average (Figure 1.7). Most notably, there appeared to be a dry season depression in the higher quantiles of male snakes in the limestone, scrub and *Leucaena* forest types and an increase in the ravine forest, savanna and urban habitats, potentially indicating seasonal changes in habitat use or foraging activity of large males among these habitat types. The difference between mid-quantile SVLs for male and female savanna snakes, previously noted, seems to be primarily driven by differences that occurred in the dry season, though an explanation for this pattern is not evident. Occasional site-specific abundances of small snakes as evidenced by low estimates in the lower quantiles (e.g., RAV3, SAV1, SAV3; Figure 1.3) may be evidence of recent breeding activity. However, these do not appear to be associated with season, at least when averaged by habitat type (Figure 1.7). This is not surprising in that Brown Treesnakes on Guam do not exhibit notable seasonality in reproduction (Savidge et al., 2007; Mathies et al., 2010).

Body conditions of snakes can be sensitive to recent fluctuations in prey availability which in turn may be driven by seasonal weather patterns (Madsen et al., 2006; Ujvari et al., 2011). I anticipated that the surge of primary productivity associated with increased precipitation during the wet season would lead to an increase in insects, in turn feeding the small lizards that have become the primary prey of Brown Treesnakes on Guam (Savidge, 1988); this would result in overall better body conditions for snakes in the wet season. Overall, season did not appear to have a consistent effect on snake body condition in general, with only a marginal increase in the highest quantiles of CI for male snakes (Figure 1.6). More distinct variation in CI was evident when observing seasonal changes by habitat (Figure 1.8). In general, wet season estimates for both sexes and all habitats tended to conform to the quantile means, while the greatest deviations occurred during the dry season. The previously-mentioned patterns in limestone forest and urban CI appear to be primarily driven by CI extremes occurring during the dry season.

Other published accounts of geographic or ecological variation in population characteristics of snakes have demonstrated variation in: sexual size dimorphism (*Morelia spilota*, Pearson et al., 2002a,b; *Elaphe quatuorlineata*, Filippi et al., 2005); body size, age structure, and proportions of reproductive animals (*Notechis* spp., Shine, 1987; *Opheodrys oreganus*, Plummer, 1997; *Liasis fuscus*, Madsen and Shine, 1998; *Elaphe obsolete*, Blouin-Demers et al., 2002; *Natrix natrix*, Luiselli et al., 2005; *Crotalus oreganus*, Jenkins et al., 2009); reproductive characteristics of females (*Thamnophis sirtalis*, Gregory and Larsen, 1993; *Hierophis viridiflavus*, Zuffi et al., 2007; *Vipera aspis*, Zuffi et al., 2009); individual growth rates (*Thamnophis sirtalis*, Bronikowski, 2000); and microhabitat use (*Corallus grenadensis*, Henderson et al., 1998). However, many of these studies occurred over very large geographic expanses (e.g., Gregory and Larsen, 1993; Blouin-Demers et al., 2002), over distinct elevational gradients (Bronikowski, 2000), or at a small number of sites (Henderson et al., 1998; Plummer, 1997). Within the snake literature, our study is one of the first to use a controlled and balanced sampling design methodology to explicitly assess the amount of variability present among replicates, habitat types, and seasons over a relatively small geographic expanse.

Spatial patterns of snake populations reflect behaviors and interactions of individuals and spatial arrangements of prey and other resources (Gregory et al., 1987). However, generalities concerning the process of habitat selection remain largely based upon conjecture (Reinert, 1993), and much remains to be learned about the influence of local ecological conditions on the spatial structure of populations. My results explore the level of variability within and among habitat types throughout the entire extralimital range of this costly invasive predator. Although I found ecological influences on size distributions and body conditions, microgeographic variation – as observed among replicates – often exceeded the level of variability observed among habitat types. Indeed, some deviations at single replicates were extreme enough to appear to drive habitat "patterns," even when the other replicates were relatively average in size or body condition distributions. Conversely, averaging over replicates ex-

hibiting much variability may suggest that the habitat is relatively average, masking what may be important variation (e.g., female SVL at ravine replicates). Such decisions to pool or parse data from multiple geographic locations may result in differing conclusions. For these reasons I maintain that a thorough understanding of the population ecology of this and similar organisms should include sampling at multiple sites within habitat types and an explicit consideration of the variability within and among habitat types, particularly for factors with strong demographic or management consequences.

The single individual characteristic most important to modulating the behavior, ecology, and management challenges associated with invasive Brown Treesnakes is body size. This characteristic drives prey utilization (Savidge, 1988; Chapter Three), susceptibility to control tools (Rodda and Reed, 2007), reproductive maturity (Savidge et al., 2007), and may be associated with patterns of habitat use and prevalence of various size classes in habitat types (Savidge, 1991; Chapter Two). As "capital breeders" (Bonnet et al., 1998), Brown Treesnakes must amass sufficient metabolic energy stores prior to successful reproduction (Naulleau and Bonnet, 1996). My results indicate significant variability in body size and body condition by site, habitat, and season which may be useful to managers planning and prioritizing intervention activities.

Despite the general trends observable in the results, this study indicates a high level of heterogeneity in population characteristics of invasive Brown Treesnakes throughout Guam; averaging population characteristics over larger areas would mask much of that heterogeneity. It appears that, with respect to prospects for snake suppression, there is little ability to make predictions about population characteristics in unsampled areas. Rather, knowledge of the possible range of population characteristics should be taken into account when making predictions about the outcomes of management interventions. Simulated suppression scenarios should incorporate the full range of variability reported here and explicitly consider sensitivity of predicted outcomes to plausible extremes of starting conditions.

There is no single "proper" scale for ecological studies (Levin, 1992) and those that include

inference from multiple scales will inherently be more robust. Particularly with respect to species with demographic strata that vary in ecological requirements such as habitat or prey types, efforts should be made to assess and account for how those strata are distributed within a heterogeneous environment. Failure to consider microgeographic variability in population characteristics of such species may result in imprecise or erroneous inference or sub-optimal management outcomes.

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Chapter Two: Ontogenetic Shifts in Invasion Risk and Microgeographic Variation in Management Classes of Brown Treesnakes on Guam

Introduction

Life history stages of invasive species may exhibit differences in relative risks and challenges associated with their management (Sakai et al., 2001). Proportions of individuals in each life history stage may vary throughout the organism's range based upon population dynamics and local ecological characteristics. Accounting for variability in population characteristics of management interest associated with local ecological differences may help optimize plans for management.

The invasion of Guam by the Brown Treesnake (*Boiga irregularis*) serves as a stark example of ecological and economic devastation wrought by a single introduced predator, particularly in vulnerable island ecosystems (Savidge, 1987; Fritts and Rodda, 1998; Rodda and Savidge, 2007). Management interventions have primarily focused on preventing spread of this predator to other vulnerable Pacific islands such as the Commonwealth of the Northern Mariana Islands and the state of Hawaii. Beyond interdiction, management objectives for Brown Treesnake populations on Guam include recovery of key habitat for re-introduction of native wildlife and reduction or elimination of other impacts on Guam's economy, ecology, and society (U. S. Fish and Wildlife Service, 2009). Landscape-scale suppression of Brown Treesnakes on Guam would meet these aims by reducing or locally eliminating populations. However, little is known about how population characteristics of Brown Treesnakes vary across Guam's landscape. To inform plans for landscape-level suppression, I sought to characterize how Brown Treesnake populations vary in demographic fractions associated with elevated management risk throughout the geographic expanse of Guam.

The two most important individual characteristics associated with Brown Treesnake demography are body size and sex. From hatching to full maturity, a Brown Treesnake may undergo a six- to seven-fold increase in body length and a 400-fold increase in body mass

(Figure 2.1). These changes can have marked consequences for nearly all aspects of an individual's natural history throughout its ontogeny, including locomotion, thermoregulation, microhabitat use, predation, optimum prey size, and vulnerability to control technologies.

Figure 2.1: Snakes of different sizes pose different invasion and management risks. In this image, the 450-mm juvenile snake is less susceptible to rodent-baited control tools and is more likely to be accidentally transported in cargo. The mature 1250-mm snake is more likely to prey on larger native prey species and poses more risk for establishment of a new population by reproduction. (Photo: S. Siers)

Changes in size also affect relative risks associated with different sizes of snakes. Smaller snakes are limited to small prey and rarely forage or move terrestrially (Rodda and Reed, 2007; Chapter Three). Larger snakes can take a wider variety of prey items, including larger native fauna and domestic animals (Savidge, 1988; Chapter Three), and are more inclined to forage terrestrially (Rodda and Reed, 2007; Chapter Three), which may lead to more movement among habitat fragments (Chapter Five) and movements into savanna and urban habitats where larger snakes are more often observed (Savidge, 1991; Chapter One). Larger snakes are also more likely to inflict medically significant bites to infant humans (Fritts et al., 1990; Fritts and McCoid, 1999). Males attain larger body size (Savidge, 1991; Chapter One), so size-related impacts may be more severe with larger male snakes, and large females have

the greatest immediate per capita reproductive potential. Due to a distinct ontogenetic shift from ectothermic to endothermic prey (Savidge, 1988; Greene, 1989; Mackessy et al., 2006), rodent-baited control methods such as traps (Rodda et al., 1999a) or toxicant-laden baits (Savarie et al., 2001; Lardner et al., 2013) are largely ineffective against snakes < 700 mm snout-vent length (SVL) and partially effective against snakes from 700 – 900 mm (Rodda et al., 2007). However, larger snakes, which are attracted to mammalian prey, are susceptible to these methods.

Reproductive status of Brown Treesnakes is most practically estimated by SVL benchmarks established by Savidge et al. (2007), who found that 95% of female snakes matured between lengths of 910 and 1025 mm, while males matured from 940 to 1030 mm. Based upon size and sex characteristics and associated differences in management risk, I designate three classes for invasive Brown Treesnakes based on sex and SVL: 1) "juvenile," snakes of both sexes measuring under 700 mm SVL, which are refractory to rodent-based attractants and baits and completely sexually immature; 2) "transitional," snakes from 700 mm to 1025 mm (females) or 1030 mm (males), of intermediate attraction to rodents and sexual maturity; and 3) "mature" snakes, fully vulnerable to rodent-based attractants and baits, with subclassifications of "mature males" (with greater possible impacts due to larger size potential) and "mature females" (with greater reproductive potential) (Figure 2.2). Management risks and challenges associated with these size classes vary based upon which stage of the invasion process is being considered (Table 1). In general, the juvenile class is most refractory to existing control technologies (Rodda et al., 2007; Lardner et al., 2013) and accidental transport (Vice and Vice, 2004), and may be less likely to be immediately discovered or reported if transported to a novel environment. Due to their large size, mature males pose highest risk for impacts such as predation on an expanded size distribution of native and domestic fauna and serious bites to humans – while mature females, particularly gravid or sperm-storing females, pose highest risk for establishment of new populations after accidental transportation to a snake-free island.
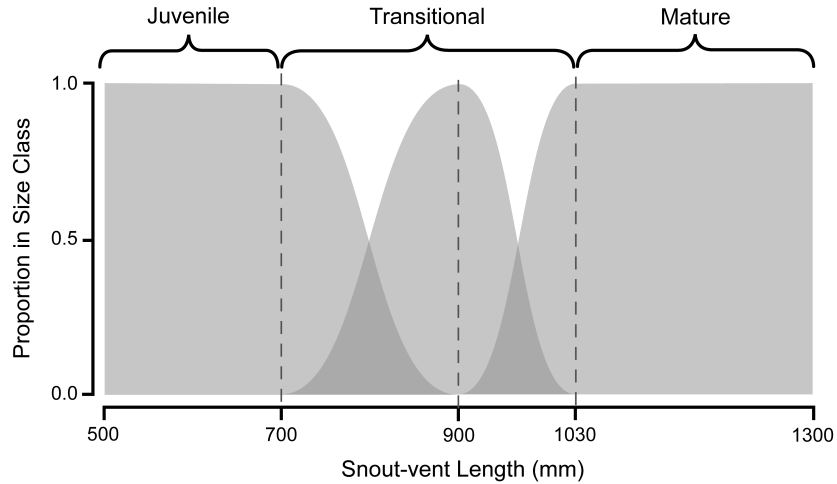
Figure 2.2: Idealized representation of the transition from juveniles, which are refractory to rodent-based control tools, to intermediately-targetable but immature snakes, then to mature snakes which are vulnerable to rodent-based control tools. Maturation sizes vary slightly for males and females, as discussed in the text (Savidge et al., 2007).

My objectives for this study were to assess variability of relative distributions of snakes among these size classes across Guam's landscape, determine whether these distributions were affected by local ecological conditions (i.e., habitat type), and interpret these results from the standpoint of consequences for interdiction and suppression of Brown Treesnakes on Guam. To achieve these aims, I established a comprehensive, habitat-stratified sampling protocol spanning the geographic expanse of Guam covering the entire known extralimital range of this species.

Table 2.1: Ontogenetic shift in invasion risk by management classification throughout the invasion process. Juvenile = both sexes < 700 mm SVL; transitional = 700 to 1025 mm (females) or 1030 mm (males); mature = females > 1025 mm and males > 1030 mm. Citations: [1]Vice and Vice, 2004; [2]Rodda et al., 2007; [3]Savidge et al., 2007; [4]Rodda and Reed, 2007; [5]Savidge, 1988; [6]Savidge, 1991; [7]Chapter Five; [8]Fritts and McCoid, 1991; [9]Fritts and McCoid, 1999; [10]Fritts et al., 1990; [11]Lardner et al., 2009a [12]Lardner et al., 2013; [13]Savidge, 1987; [14]Rodda et al., 2008, [15]Rodda and Fritts, 1992; [16]Chapter One; [17]Chapter Three.

Invasion Stage	Juvenile Risk	Transitional Risk	Mature Risk
TRANSPORT: Risk associated with being moved to a new location.	HIGHER: Small snakes disproportionately found in outbound cargo.[1] Invulnerable to port protection traps and toxicant baits that filter out larger snakes. [2]	MODERATE: Most numerous size class of snakes (Figure 2.4) but moderately susceptible to interdiction tools.	LOWER: Adult snakes are more likely to be trapped before reaching cargo or detected by cargo inspectors.
ESTABLISHMENT: Risk of founding a new population upon being transported.	LOWER: Very small snakes are not yet reproductively mature[3] and may experience higher mortality before maturation.	MODERATE: Contains some reproductive individuals, with non-reproductive snakes approaching maturation.	HIGHEST: Reproductively mature females have the highest per capita reproductive potential. A single gravid female may establish a new population. Males may pose only moderate risk, with per capita risk decreasing at higher densities as mature females become the rate-limiting stratum.
LAG: Risk of not being observed or not prompting reaction in new location.	HIGHER: Smaller size classes are harder to detect[2] and less likely to leave arboreal habitats[4], and thus less likely to be observed by the public and reported.	MODERATE: Relatively more observable, more likely to be reported to a management agency.	LOWEST: More likely to be found in urban habitats,[5,6,16] more likely to be noticed and reported, particularly very large males.
SPREAD: Risk of moving from introduction site to new location.	LOWER: Very small snakes appear to move less, particularly across terrestrial gaps, e.g., roads.[4,7]	MODERATE: More vagile than smaller snakes.	HIGHER: More likely to move longer distances, more likely to forage away from forest habitat.[6,7] May form the invasion front, spreading as local supplies of large prey are depleted.[13,14]
IMPACT: Risk of ecological or economic damages associated with invasion.	LOWER: Prey only on small lizards,[5,17] low per capita impact. Too small to pose any risk to humans or domestic animals. Impact only higher with high densities on sensitive prey species.[15]	MODERATE: Wider range of prey take, e.g., eggs of smaller birds.[5,17] Most abundant size range (Figure 2.4), with impact increasing with density.	HIGHEST: Larger snakes take a much wider range of prey sizes,[17] including native threatened and endangered fauna[5] and domestic animals,[5,8,17] and inflict bites on humans[9] and their infants,[10] particularly male snakes which achieve much greater sizes.[16]
CONTROL: Risk of evading effective targeting by interdiction or suppression tools.	HIGHEST: Almost completely impervious to rodent baits, which includes all major control tools,[2,11,12]	MODERATE: Increasingly trappable with size, rodent baits nearly fully effective by 900 mm SVL.[2]	LOWER/HIGHER: Rodent baits fully effective; however, any failure to effectively target gravid females poses high risk of control failure due to perpetuation of reproduction.

Methods

Habitat stratification and site selection – I selected three study sites within each of six habitat types as classified by Liu and Fischer (2005) following the nomenclature of Mueller-Dombois and Fosberg (1998). Sites were dispersed across Guam's and contained large uninterrupted tracts representative of respective habitat type. Limestone forest (LIM; 13% of Guam's land cover) is characterized by moist, broadleaved evergreen forest of predominantly native species on elevated limestone plateaus. These habitats are critical to preservation and recovery of Guam's native flora and fauna. Ravine forests (RAV; 8%) are low-lying areas surrounding flowing and ephemeral rivers, and are primarily moist green forests containing higher proportions of palms, bamboos, and *Pandanus*. Scrub forest (SCR) is a variable forest comprised primarily of secondary growth of non-native species resulting from a long history of human disturbance. It is the most extensive class of forest on Guam, covering 23% of Guam's land mass and comprising 58% of forest cover. *Leucaena* stands (LEU; 3%) are primarily comprised of *Leucaena leucocephala* ("Tangantangan"), an introduced species often used in many parts of the world to cover deforested areas. *Leucaena* provides excellent habitat for several introduced species, including Brown Treesnakes. Nearly all forest formations on Guam have some amount of *Leucaena*, particularly at forest edges; however, in some areas it forms nearly monotypic stands. Savanna complex (SAV) is characterized by a mosaic of predominant grassland with emergent shrubby vegetation and erosion scars, and comprises a significant proportion of Guam's southern region (21% of Guam's total area). While snake densities are presumed to be lower in savanna than in forest habitat, they are still likely higher than most native range habitats and require consideration in any landscape-level suppression or eradication effort. Urban areas (27%) are diverse and include industrial, commercial and residential areas. For reasons of consistency, access, and public awareness, I elected to concentrate our surveys in and around urban residential (URB) areas. These six habitat types comprise 95% of Guam's land cover (Figure 2.3).

Survey methods – We employed visual surveys to sample snake populations at the 18
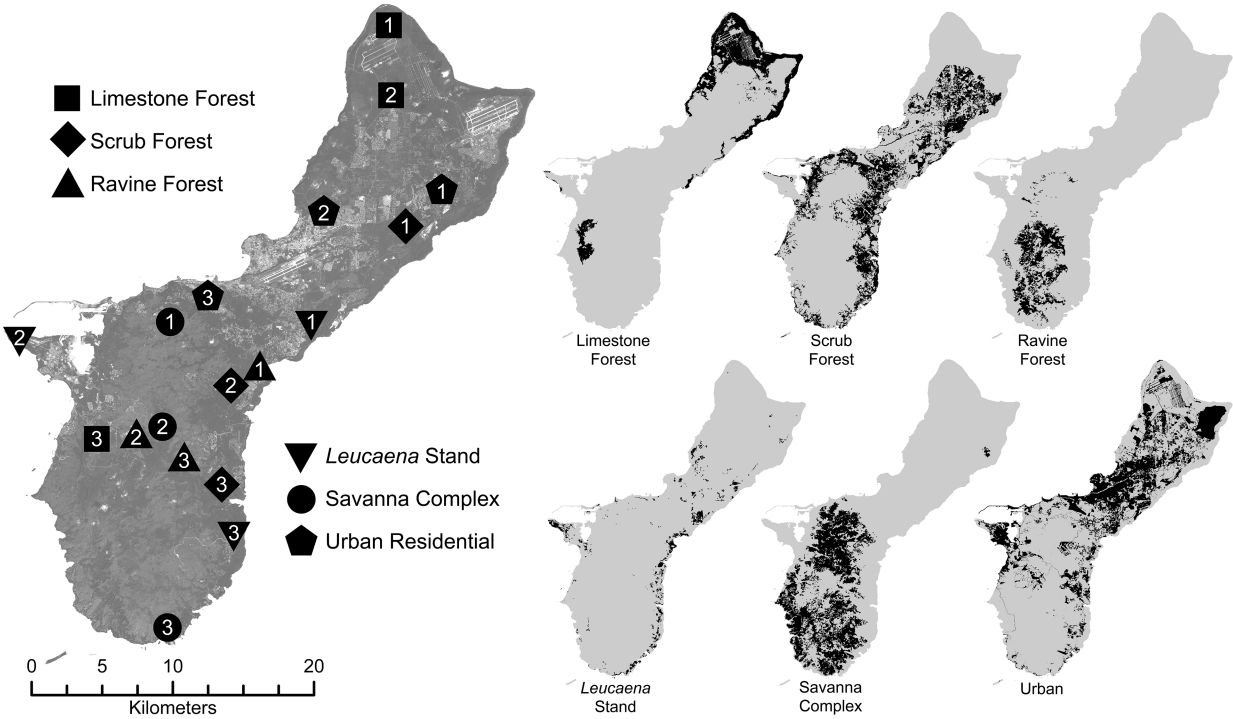
Figure 2.3: Habitat classification map of Guam depicting the distribution of the six target habitat types (Liu and Fischer, 2005) and 18 sample locations. Symbols represent habitat types and numbers refer to the respective replicate (1-3) within that habitat type.

selected sites. Visual surveys provide low yield per unit effort when compared to trapping, but provide samples that are more representative of the population and with less size bias (Rodda et al., 2007). We commenced surveys at sunset and searched for snakes for three to four hours, including much of the peak activity period of Brown Treesnakes (Rodda et al., 1999b). Trained searchers were equipped with powerful headlamps with beam characteristics that optimize detection (Lardner et al., 2009b). Searchers followed habitat edges at a slow pace, roughly 0.5 km per hour, examining visible vegetation and non-vegetative structure for presence of snakes. We surveyed most forest habitats from road edges. Savanna searches included road edges, footpaths, and trackless searches throughout the habitat mosaic, including edges of erosion scars. We conducted urban surveys by searching structures and vegetation in residential yards, with all yards separated from large forest tracts by at least one paved road. Searchers stopped searching when encountering habitat formations inconsistent with search objectives and resumed searching upon returning to representative

habitat.

Sampling objectives – To obtain enough data to accurately describe size distributions, I selected a target sample of 100 snakes from each of the 18 sites. To minimize bias resulting from short-term population dynamics or seasonal effects, I balanced sample sizes between the wet season (June through November) and dry season (December through May), with at least two quarterly bouts per season. The one exception to this sampling scheme was the second limestone forest replicate (LIM2), which was sampled in one relatively continuous effort due to impending closure of a snake-proof barrier constructed at the site; at this site, 90 snakes were collected in the wet season and 10 in the dry season.

Snake processing – Upon visual detection, snakes were hand-captured. Surveyors recorded time and location, microhabitat data, and morphometric data including snout-vent length (SVL) obtained by stretching the snake along a flexible tape ruler, and weight using Pesola spring scales (Pesola AG, Baar, Switzerland). Captured snakes were transported to the U.S. Geological Survey Brown Treesnake Lab the following morning, where SVL and weight were re-measured to validate field data. Snakes were then euthanized and necropsied. We determined sex by examining internal reproductive anatomy. All animal use was conducted in accordance with Colorado State University IACUC Protocol #09-1436A.

Management classification – I assigned snakes to one of six management classes: females and males of three developmental stages (juvenile, transitional, and mature) based on SVL classes as described above. I depicted distributions of snakes among management classes by site and habitat groupings in a box plot and tested by comparing observed counts within management categories to those expected by pooling all other sampled sites as a reference distribution using Fisher's exact test. Separate Chi-square tests were used to assess significant deviation from 1:1 sex ratios.

I assessed regional similarities in proportions within management classifications by hierarchical clustering (average method based on a correlation distance matrix) using the R package 'pvclust' (Suzuki and Shimodaira, 2013), which produces a clustering dendrogram

with approximately unbiased P-values for clusters via multiscale parametric bootstrap re-sampling. I plotted cluster nodes on a map to visually assess relative geographic aggregation or non-aggregation of sites within clusters.

Site-level and habitat-level heterogeneity in prevalence of 1) attractant-refractory juveniles of both sexes, 2) high-impact mature males, and 3) mature females of high establishment risk were independently tested by assessing fit of models describing alternative hypotheses associated with various site identity and habitat classification schema using logistic regression. The binary response variable was whether a given snake did or did not belong to the management class of interest. The term *site* assigned a categorical covariate for each of the 18 sites, allowing full site-by-site variability in estimation of prevalence – or, proportion of population – for each of the response variables (management classes). The alternate classification *hab* pooled all sampled snakes into one of six nominal habitat types (limestone forest, scrub forest, ravine forest, *Leucaena* stand, savanna complex, and urban residential) to assess whether prevalence varied by habitat type. Other alternatives included *fsu* which lumped four forest types (limestone, scrub, ravine, and *Leucaena*) into one forest classification and left savanna and urban habitats to vary independently (three habitat classes), and *frsu* which pooled limestone, scrub and *Leucaena* forests, but allowed ravine forest to vary independently along with savanna and urban habitats, based on *a priori* observations of field observers that ravine forest searches tended to produce lower rates of snake captures than in other forest habitats and snakes that were in poorer body condition (four classifications). Additionally, as I balanced sampling between wet and dry seasons, the term *seas* was investigated for additive and interactive effects of season on prevalence of snakes in respective management classes. The higher-order or "global" model for this assessment was:

$$logit(\pi) = \beta_0 + \beta_{[class]} + \beta_{seas} + \beta_{[class]*seas} \tag{1}$$

where π is the estimated proportion, or prevalence, of the respective management class, and [*class*] is one of the four habitat classification alternatives (*site*, *hab*, *fs*u or *frsu*). All

possible nested combinations of terms were considered, resulting in a set of 14 candidate models, including season-only and intercept-only (single prevalence estimate) models. I conducted logistic regression using R version 3.0.2 base function 'glm' (family = "binomial") (R Core Team, 2013). Model selection was based upon an information-theoretic approach (Akaike's Information Criteria corrected for small sample size, AICc).

Results

We captured a mean of 100 snakes (n = 99 to 104) at each of the 18 study sites, for a total of 1804 snakes (990 males and 814 females). Juvenile snakes (<700 mm) constituted 38.2% of the sample, transitional snakes accounted for 48.8%, and the remaining 13.0% (8.3% males, 4.7% females) were mature per size criteria of Savidge et al. (2007). Qualitatively, size distributions in Figure 2.4 depict a concentration of snakes in the 500 to 1000 mm size range with a long right tail in larger size classes, particularly for males.
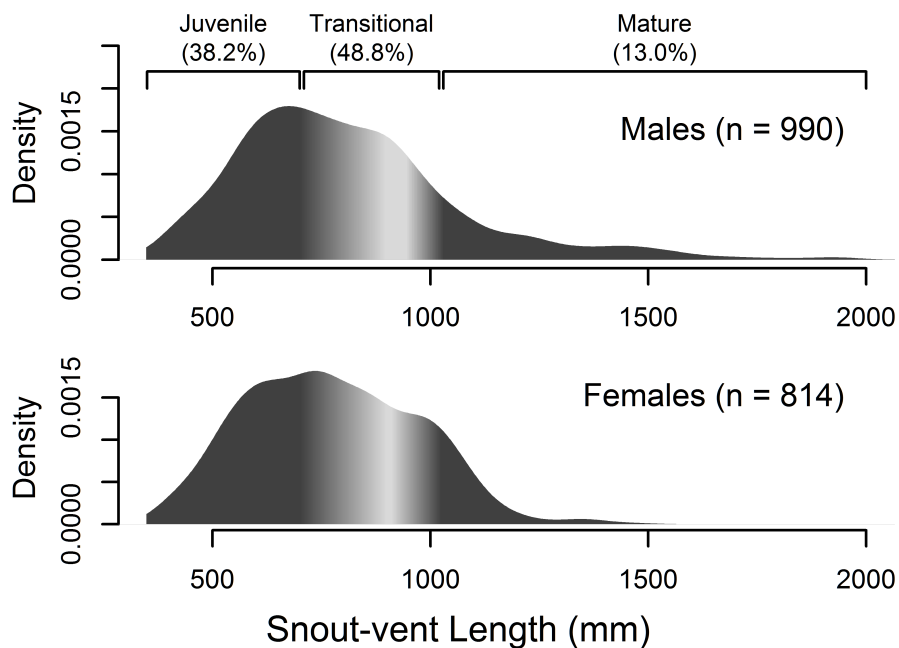

Figure 2.4: Size distributions of male and female snakes sampled at all 18 sites. Note the longer right tail for larger male snakes. Shadings in the "transitional" class represent the shifts toward trappability at the lower margin and maturity at the upper margin.

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The distribution of sampled snakes among different management classes varied significantly within and among habitat types (Figure 5). Overall, most sites yielded a large proportion of transitional snakes with considerable variability among sites in proportions of juvenile and mature snakes. Ten of 18 sites exhibited significant variation when compared to all other sites pooled, including all three savanna sites. When samples were pooled by habitat type, all types exhibited significant variation from management class distributions of all other sites combined, suggesting variation in distribution among management classes was influenced by ecological differences among habitat types.

Similarly, sex ratios were highly variable. Overall there was a significant male sex bias at 1.22:1 for the entire sample (n = 1804, P < 0.001). Only the RAV1 site showed a significant female bias. *Leucaena* stand habitat displayed the strongest and most consistent trend, with all three replicates showing highly significant male biases.

Hierarchical clustering elucidated similarities among sites in distributions of snakes among management classes (Figure 2.6). The urban site URB2 (node "A") and ravine forest site RAV3 (node "B") were distinct outliers, likely owing to respectively larger and smaller proportions of mature individuals in samples (Figure 2.5). Node "C" was comprised of four sites and included two geographically proximate limestone forest sites at the northern end of the island. Node "D" contained six sites with a mix of four habitat types; SCR1 and SCR3 were clustered closely, and while URB1 and URB3 were under the same node they were in separate sub-clusters. The remaining node, "E," demonstrated the most apparent clustering by habitat type, including all three *Leucaena* replicates and two remaining savanna sites clustered closely together. Examination of geographic distributions of sites by clustering node (Figure 2.6) indicates little appreciable pattern in geographic distribution of similarity in management class composition.
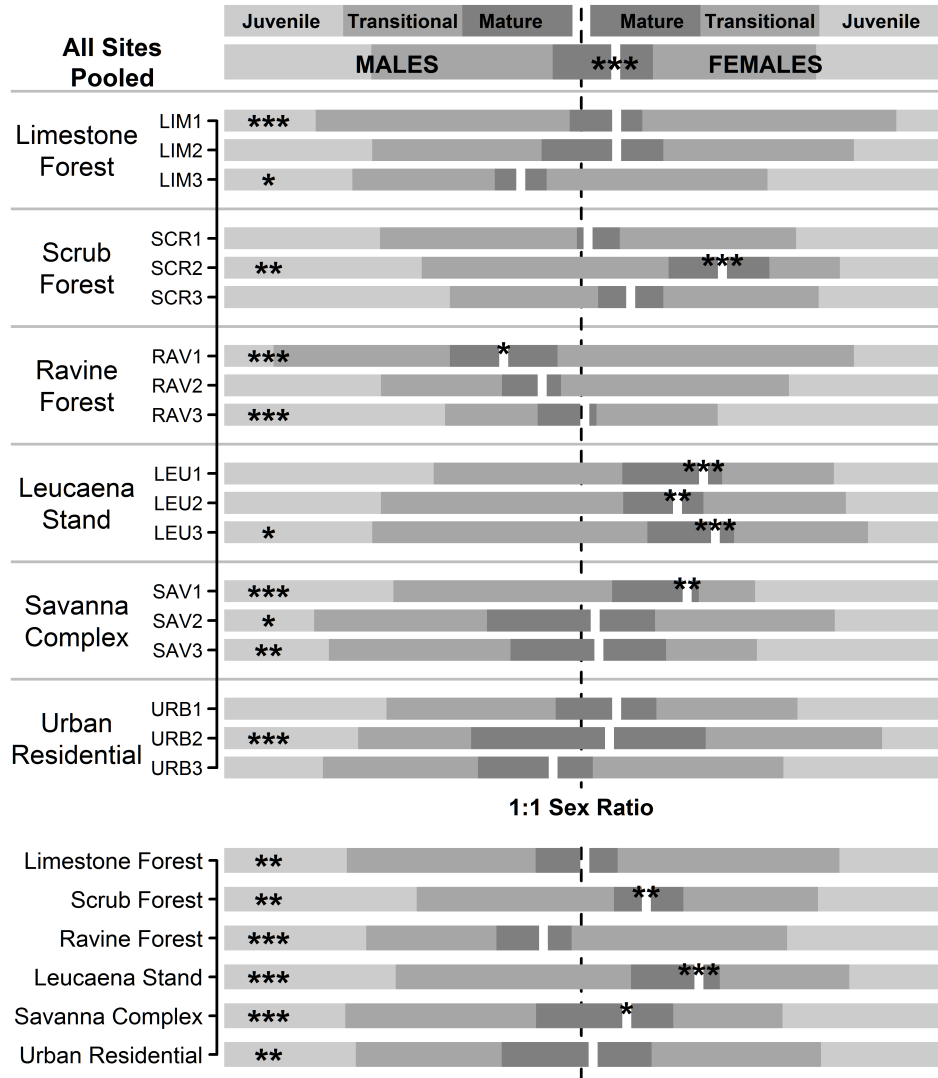
Figure 2.5: Breakdown of site samples by proportions of Brown Treesnakes in management classes, with the bottom six bars representing pooling of sites by habitat type. Asterisks on bars at left indicate significance of differences from the distribution of all other sites combined, excluding the one tested. The vertical dashed line indicates a 1:1 sex ratio, and asterisks between the bars of sexes indicate a significant sex bias. P-values: "*" < 0.05, "**" < 0.01, "***" < 0.001.
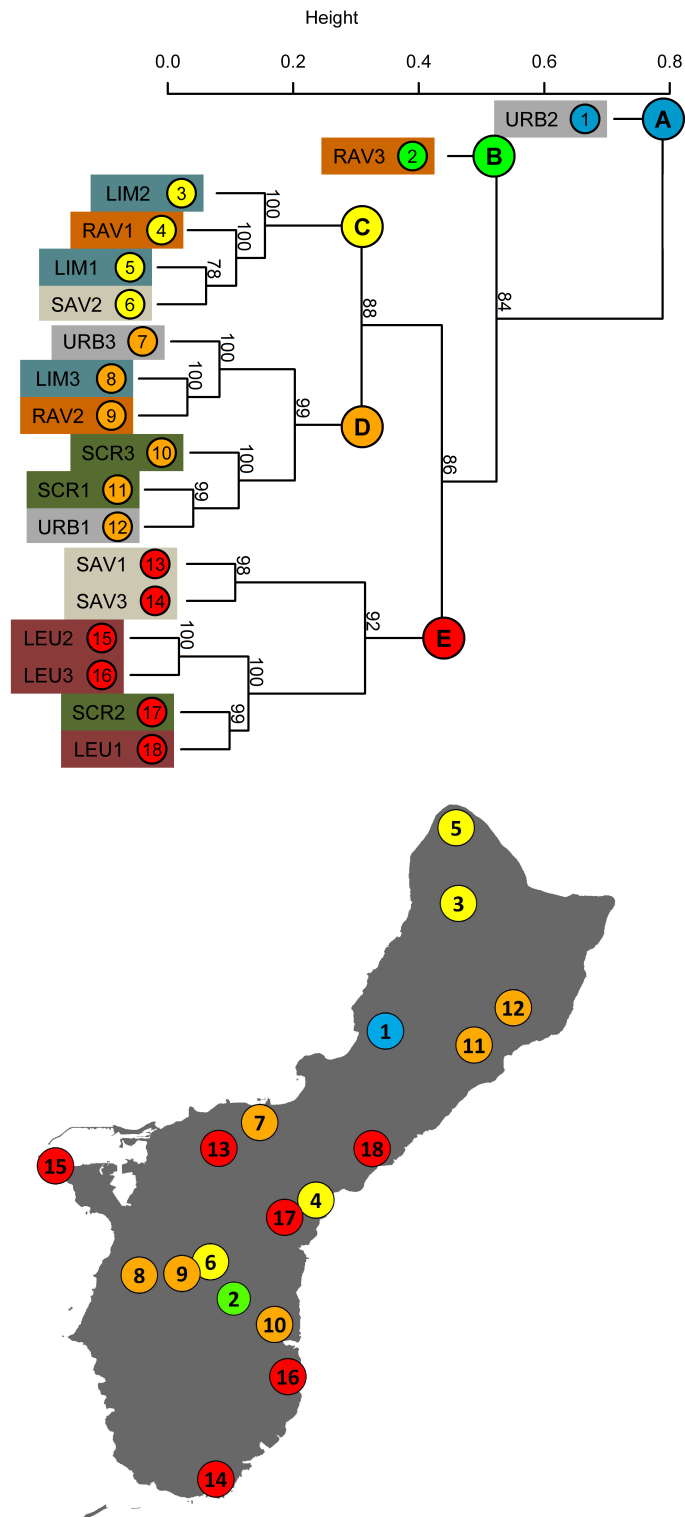
Figure 2.6: Dendrogram depicting clustering of sites by similarities in distribution of sampled snakes among management classes, with corresponding locations indicated on the map. Gray values on dendrogram edges denote approximately unbiased P-values, with high values (e.g., 95%) strongly supported. LIM = limestone forest, SCR = scrub forest, RAV = ravine forest, LEU = *Leucaena* stand, SAV = savanna, URB = urban.

Logistic regression revealed that site-by-site variation in prevalence of juvenile Brown Treesnakes far outweighed any effect of habitat type (Table 2). The top habitat model for juveniles (*hab*) outperformed the intercept-only model by 3.29 AICc units, indicating some contribution of habitat type, but the site model outperformed the habitat model by 53.47 AICc units, indicating an overwhelming amount of inter-site variability beyond any habitat effect. Both forest-aggregated classifications (*for*1 and *for*2) performed more poorly than the intercept-only model. Thus, variability within habitat types precludes us from making any predictions about prevalence by habitat type, as can be graphically interpreted from Figure 2.7a. The top model carried 41.8% of model weights, while the remaining 58.2% of weights went to other models including the *site* term. The second model, carrying 40.4% of the model weights, included *season* and *season ∗ site* interaction terms along with the *site* term; however, the effect of season was non-significant (P = 0.677) and appeared to be included only because of the significant increase in juvenile snakes at the URB2 site during the wet season (P = 0.006).

Table 2.2: Results for the full logistic regression model set for respective management classes of snakes. Top model ΔAICcs and weights are in bold. Results that ranked more poorly than the intercept-only model are italicized. K = number of model parameters. Habitat classification schema: *site* = separate term for each of the 18 sites surveyed; *hab* = six habitat classes; *fsu* = three levels, forest, savanna or urban; *frsu* = four levels, forest, ravine, savanna or urban. *seas* = season (wet or dry).

| Model | K | Juveniles | | Mature Males | | Mature Females | |
		ΔAICc	Weight	ΔAICc	Weight	ΔAICc	Weight
site + seas + site ∗ seas	36	0.07	0.404	*24.49*	*0.000*	23.09	0.000
site + seas	19	1.71	0.178	9.41	0.004	*7.51*	*0.009*
site	18	**0.00**	**0.418**	10.04	0.003	5.83	0.022
hab + seas + hab ∗ seas	12	54.22	0.00	2.81	0.118	*12.04*	*0.001*
hab + seas	7	55.23	0.00	2.96	0.109	4.97	0.034
hab	6	53.47	0.00	4.34	0.055	3.15	0.083
for2 + seas + for2 ∗ seas	8	56.22	0.00	4.00	0.065	*8.49*	*0.006*
for2 + seas	5	*61.49*	*0.00*	5.58	0.029	3.76	0.062
for2	4	*59.98*	*0.00*	6.86	0.016	1.89	0.157
for1 + seas + for1 ∗ seas	6	56.07	0.00	**0.00**	**0.480**	4.62	0.040
for1 + seas	4	*60.89*	*0.00*	3.61	0.079	1.87	0.158
for1	3	*59.44*	*0.00*	4.92	0.041	**0.00**	**0.403**
seas	2	*58.24*	*0.00*	18.80	0.000	*7.97*	*0.007*
Intercept	1	56.76	0.00	19.59	0.000	6.19	0.018
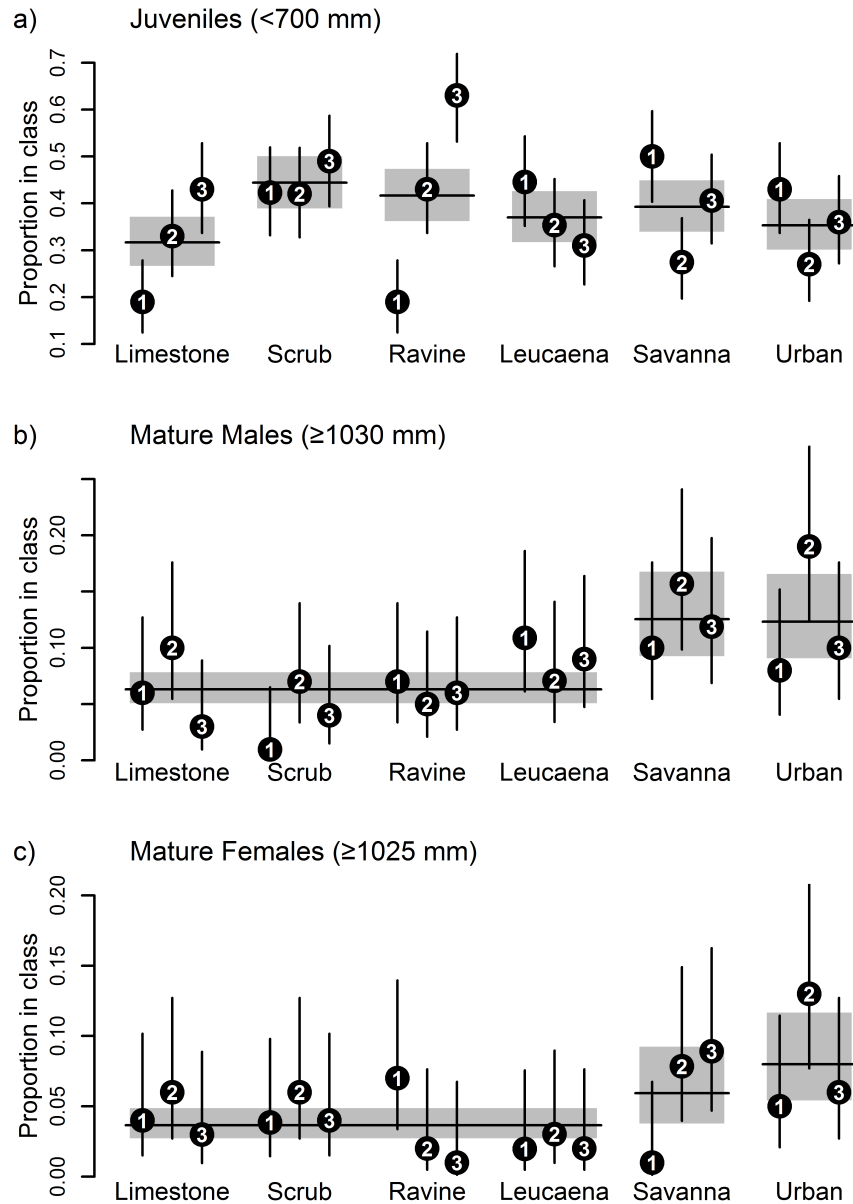
Figure 2.7: Site estimates for proportions of snakes in respective management classes, with vertical lines representing 95% confidence intervals for the estimate. Numbers in points refer to the replicate number, with locations referenced in Figure 2.3. Intra-site variability in proportions of juveniles exceeded variation among habitats, but habitat variability (indicated by shaded 95% confidence limit boxes and horizontal mean estimate lines) was still significant when compared to an intercept-only (average) model. Variation in mature males and females was best described by the model that aggregated all forest types into one category.

The top model for mature male snakes ($fsu + seas + fsu * seas$) indicated significantly higher prevalence in savanna and urban habitats and lower prevalence in forest habitats

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pooled under a single estimate, suggesting little variability among forest habitat types and not enough intra-habitat variability among replicates to warrant inclusion of a term for each site (i.e., there was more variability among these three habitat types than among replicates within them; Figure 2.7b). The fsu term was included in models carrying 60% of model weights. The next-highest scoring habitat classification was hab, with the top habitat model outperforming the intercept-only model by 16.78 AICc units, indicating significant variation by habitat but not enough to warrant breaking four forest types into separate classes. An effect of season was present in models carrying 89% of AICc weights, with model-averaged coefficients indicating a significant increase in prevalence of mature males in samples during the wet season, though interaction terms indicated this was much more pronounced in forest habitats and male prevalence was lower in savanna and urban habitats during the wet season.

Variation in prevalence of mature females in samples was best described by pooling forest habitat types, with the top model containing only the fsu term. The among-habitats variation model (hab) still outperformed the intercept-only model, but only by 3.04 AICc units. Similar to mature males, the overall forest prevalence of mature females was lower than in savanna and urban habitats (Figure 2.7c). Mature female models incorporating a $seas$ term carried only 32% of model weights, with the coefficients indicating fewer mature females in the wet season, though the P-value for this effect was non-significant.

Discussion

Landscape-level sampling revealed significant heterogeneity in distributions of sampled snakes among respective management classes (Figure 2.5). While variability among replicates within habitat types is considerable (e.g., ravine forest replicates), when samples were pooled by habitat type, each habitat type differed significantly from the reference distribution generated by pooling all other habitats. However, the nature of this variability does not suggest any obvious hypotheses regarding ecological or population processes driving many of these differences, with the exception of larger snakes being found in savanna and urban habitats.

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Although Rodda et al. (1999c) reported that historical samples have not deviated from a 1:1 sex ratio in any meaningful way, the male-biased sex ratio we observed (1.22:1) was consistent with the observations of Savidge (1991) wherein she found a 1.44:1 male bias in a sample of 897 snakes collected between 1980 and 1987. Our sex bias result, based upon more systematic and finely-stratified sampling, indicates that while there is significant variability among sites within habitat types, some habitat types (scrub forest, *Leucaena* stand, savanna) do demonstrate a significant sex bias, at least over the one- to two-year span of our sampling efforts. Whether this sex bias is real (e.g., due to more male hatchlings or higher mortality of females) or perceived due to higher detectability of male snakes (e.g., Christy et al., 2010), there are not likely meaningful consequences for management efforts as there is little evidence for sex biases in effectiveness of control tools.

Proportions of juveniles are highly variable and unpredictable on the basis of habitat type. Considerable site-by-site variation exists, with predicted proportions ranging from 19% at LIM1 and RAV1 to 72% at RAV3 (Figure 2.7a). Though proportions within scrub forest and *Leucaena* stand sites were relatively consistent, other habitat types showed more variability with both extremes of prevalence occurring in the same ravine forest habitat type. These results were strongly supported by relative model weights (Table 2). In exploratory analyses we sought a connection between mature females in good body condition and proportions of juvenile snakes found at a site, hypothesizing that more robust females may be producing more and larger clutches which might explain some of the variability in juvenile relative abundance; however, no such correlation was supported by the data. Because mature female snakes are quite vagile and may deviate from normal movement patterns while searching for appropriate oviposition sites, areas where females forage and are collected may be very different from where they are laying eggs. Gravid female Brown Treesnakes are rarely found on Guam and little is currently known about their movement or oviposition site selection behaviors.

Proportions of mature males and females were relatively consistent among forest habitat

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types and demonstrably higher in savanna and urban habitats, with highest proportions of both mature males and females at the URB2 site. Presence of more mature male and female snakes in savanna and urban habitats may be explained by a higher prevalence of large-bodied prey species, namely grassland rodents in savannas and rodents, introduced birds and poultry in urban residential areas (Savidge, 1988, 1991).

Effects of season on proportions of juvenile or mature snakes collected were relatively minor and varied among sites and habitats in ways that did not give clear indications of any causality. However, the presence of seasonal effects suggests that a thorough population characterization at a given site should be based upon samples collected in both seasons.

We found little evidence of regional similarities in distributions among management classes (Figure 2.6). The high degree of variability among replicates within habitats and broad geographic distribution of those replicates did not appear to translate to regional similarities in distributions. This result suggests little spatial autocorrelation in distributions of snakes among management classes, at least at the scale of our sampling, and a greater role for small-scale local factors (i.e., microgeographic variation) in structuring populations.

The management consequences of our results may vary with respect to particular stages of the invasion process (Table 1). At the transport stage, juvenile snakes may be of greater risk as evidenced by their prevalence in the sample of interdicted snakes in outbound cargo (Vice and Vice, 2004), possibly due to their non-attraction to rodent-based control measures protecting cargo ports; the overall high proportion of juvenile snakes on Guam suggests that efforts directed towards intercepting juvenile snakes in transportation areas is critical. Reproductively mature females, posing highest establishment risk for other areas, appear to occur at relatively low frequencies ($\sim$4%) in forest habitats, but at higher frequencies in savanna ($\sim$5.5%) and urban ($\sim$7.5%) habitats. At the lag stage of an invasion, as might occur if incipient populations are to become established in other snake-free locales such as Hawaii, small snakes are less likely to be observed, and all snakes are likely to be at low abundance at this stage. Higher prevalence of larger males and females in urban habitats,

if replicated within a new incipient population, may shorten the lag period as larger snakes in close proximity to humans will increase likelihood of reporting. Spread of an incipient snake population may be accelerated by mature males and females due to their reproductive potential and higher probability of crossing roads and other habitat gaps (Siers, Chapter Five). The impact risks associated with Brown Treesnakes are likely to increase with snake size, as transitional and mature snakes are more capable of predation on a wider range of prey species, leading to potential extirpation or extinction of native prey species as documented on Guam by Savidge (1987). Larger snakes also pose more threat to humans and domestic animals. While our results may suggest that these impacts would fall more heavily upon savanna and urban habitats than on forest habitats, it should be noted that Brown Treesnakes on Guam have already effectively extirpated forest fauna, causing a shift to a smaller proportion of mature snakes. This is not likely to be the case in a novel environment with plentiful forest prey. Finally, with respect to control, high and highly variable prevalence of snakes too small to be effectively targeted by current control technologies likely poses the single greatest threat to prospects for successful landscape-scale suppression or localized eradication of Brown Treesnakes, and this variability does not behave in any predictable manner. While adult snakes are relatively easy to target with traps or toxicant baits, failure to completely control adult females may defeat suppression objectives by allowing production of new juvenile snakes too small to be effectively targeted.

Much of the uncertainty as regards effective landscape-scale control of Brown Treesnakes lies within the transitional class of snakes. Theoretically, as smaller snakes mature, they become susceptible to rodent baits prior to becoming reproductively mature (see the transitional stage in Figure 2.2). This stage of development may pose a "window for control" during which snakes are susceptible to control but not yet reproductive. Repeated applications of trapping or toxicant delivery may target maturing snakes, potentially killing all snakes before they can reproduce. However, uncertainty remains about the relative timing of transition to targetability and on to maturation. This remains an active area for research.

In this manuscript we offer a framework for consideration of variation in management consequences associated with ontogenetic shifts in management risk by sex and size class, invasion stage, and variability of distributions among management classes within and among habitat types. While some habitat trends are significant and consistent, the overall result is one of much heterogeneity and little predictive ability. Any simulation modeling of candidate suppression scenarios must incorporate the full range of variability documented here.

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Chapter Three: Spatial, Temporal, and Ontogenetic Variation in the Foraging Ecology of Invasive Brown Treesnakes on Guam

Introduction

As with all obligate predators, the ecology and evolution of snakes are largely driven by their interactions with prey (Greene, 1983; Mushinsky, 1987). Spatial and temporal heterogeneity in availability of prey resources have the potential to exert strong influence on snake habitat use, foraging behavior, and fitness (Reinert, 1993; Forsman and Lindell, 1997; Shine and Madsen, 1997; Madsen et al., 2006). Geographic variation in diets of generalist snakes is common, and largely reflects local availability of prey (Kephart and Arnold, 1982; De Queiroz et al., 2001; Capula and Luiselli, 2002; Glaudas et al., 2008; Santos et al., 2008). Prey availability may also change temporally, by season or weather patterns (Weatherhead et al., 2003; Madsen et al., 2006; Sperry and Weatherhead, 2008; Ujvari et al., 2011) or in response to prey population trends (Andrén, 1982). Local effects on diet composition can be so pronounced that diets of sympatric species may be identical (Kephart and Arnold, 1982). Most studies of dietary variation within a species focus on large-scale geographic effects such as mainland-island comparisons (Shine, 1987) and continental gradients (Hamilton et al., 2012). However, diets can vary on much smaller scales, and pooling diets over larger areas can mask important differences at microgeographic scales (Kephart, 1982; Reinert et al., 2011) such as among habitat types (Weatherhead et al., 2009) or between urban and natural habitats (Luiselli et al., 2001).

Variability of prey resources among habitats may affect size classes of snakes differently. Body size profoundly influences an organism's ability to obtain and process resources and interactions with other organisms. Within a population of a species with indeterminate growth (e.g., fishes and reptiles), body size can range across multiple orders of magnitude. As size changes, metabolic rates, physical capabilities, feeding requirements, inter-species interactions, and survivorship may change. Resources required by juvenile animals may

be quite different than those required by larger individuals, leading to different patterns of resource use among size or age classes (Law and Dickman, 1998). Changes in "the patterns in an organism's resource use that develop as it increases in size from birth or hatching to its maximum" comprise the concept of the "ontogenetic niche" (Werner and Gilliam, 1984). The necessity of obtaining size-appropriate prey may result in differing patterns of foraging behavior or habitat use among size classes if the spatial distributions of prey vary. Among snake species, size-dependent variation in prey and habitat resource use has been frequently documented (e.g., Lind and Welsh, 1994; Madsen and Shine, 1998; Shine and Li-Xin, 2002; Weatherhead et al., 2003).

The rear-fanged colubrid Brown Treesnake, *Boiga irregularis*, is an arboreal generalist predator native to the majority of the islands of Oceania and the northern and eastern rims of Australia (Rodda et al., 1999a). Approximately 60 years after its accidental introduction to the Micronesian island of Guam (Rodda et al., 1992), it has invaded all of the island's habitat types and all native vertebrate taxa have suffered declines or complete extirpations as a result of its predation (Rodda and Savidge, 2007), including global extinction of one species and one subspecies of endemic forest birds (Savidge, 1987). The result is an impoverished vertebrate food web dominated by this hyperabundant generalist predator (Fritts and Rodda, 1998; Rodda et al., 1999b). Brown Treesnakes may undergo a six-fold increase in length and a 400-fold increase in weight throughout their ontogeny, and their use of prey and habitat resources may be different between size extremes (Figure 3.1). Previous descriptions of the feeding ecology of Brown Treesnakes on Guam have demonstrated: a pronounced ontogenetic shift in prey preferences (Savidge, 1988; Lardner et al., 2009a); an increased reliance on lizard prey (Savidge, 1988); and differences in habitat use by larger snakes resulting from the disappearance of endothermic prey from forest habitats (Savidge, 1988; Rodda et al., 1999a). However, like most descriptions of the feeding ecology of snakes and other organisms, these previous results could be augmented and improved by sampling with greater spatial, ecological, temporal, and ontogenetic specificity. Additionally, new species

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Figure 3.1: Snakes of different sizes exhibit variation in diet composition, foraging behavior, and habitat use. The ~450 mm SVL juvenile snake in this image would prey almost entirely on small arboreal lizards and spend nearly 100% of its foraging budget arboreally. The larger snake (~1250 mm) is more likely to forage terrestrially, take much larger prey including birds and mammals, and be found in habitats where larger prey species are available.

of vertebrates that are potentially suitable as prey for Brown Treesnakes have invaded Guam in the subsequent decades, with unknown effects on snake population dynamics.

Through comprehensive, stratified, and balanced sampling of foraging Brown Treesnakes, I sought to answer the following research questions: How does current diet diverge from historical and native range records, and how does diet vary within and among habitat types and seasons? What factors affect the frequency at which snakes contain recently-ingested prey? How does diet vary throughout their ontogeny and how does this variation affect the foraging ecology of different size classes? What are the roles of native and novel prey species in sustaining invasive Brown Treesnake populations on Guam?

Methods

Habitat stratification and site selection – We selected three study sites within each of six habitat types as classified by Liu and Fischer (2005) following the nomenclature of Mueller-Dombois and Fosberg (1998). Sites were dispersed across the majority of Guam's geographic extent and ground-truthed to contain large uninterrupted tracts representative of the habi-
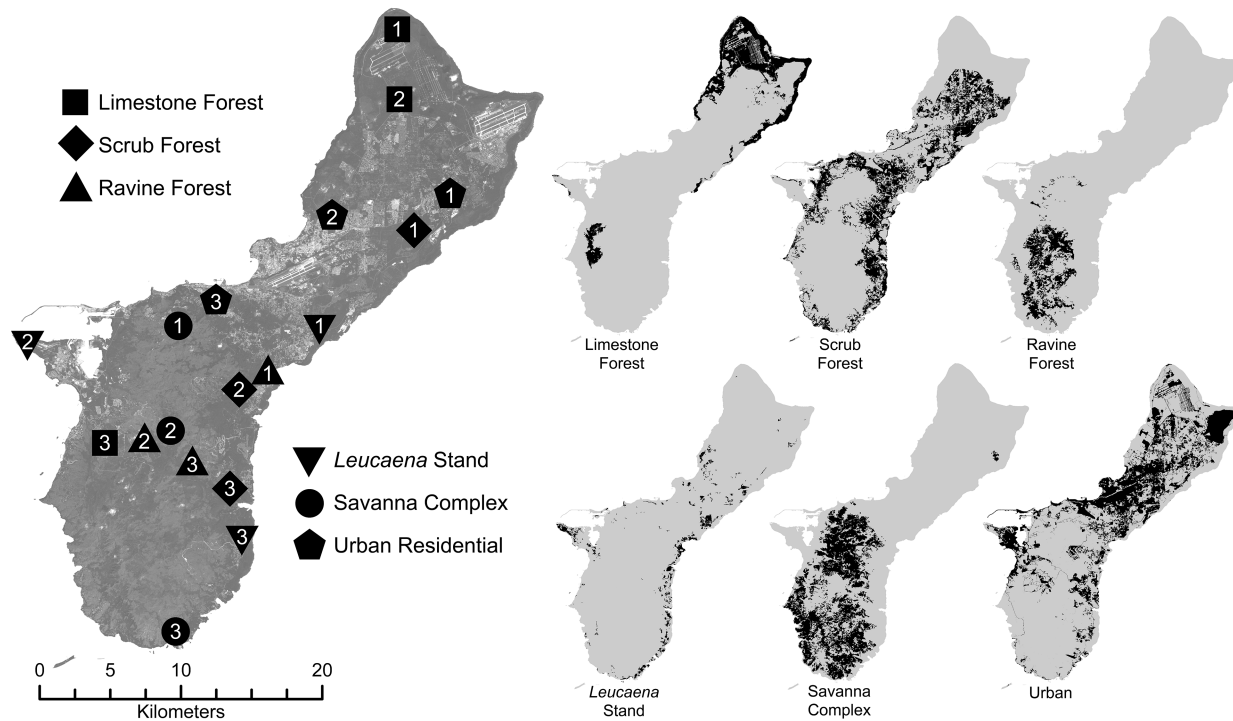
Figure 3.2: Habitat classification map of Guam depicting the distribution of six target habitat types and 18 sampling locations. Symbols represent habitat types and numbers refer to the respective replicate (1-3) within that habitat type (after the classification of Liu and Fischer, 2005).

tat type. Limestone forest (LIM; 13% of Guam's land cover) is characterized by moist, broadleaved evergreen forest of predominantly native species on elevated limestone plateaus. Ravine forests (RAV; 8%) are low-lying areas surrounding flowing and ephemeral rivers and are primarily moist forests containing higher proportions of palms, bamboos, and Pandanus. Scrub forest (SCR; 23%) is a variable forest type comprised mostly of non-native secondary growth resulting from a long history of human disturbance. *Leucaena* stands (LEU; 3%) are dominated by *Leucaena leucocephala* ("Tangantangan"), an introduced species often used to cover deforested areas. Savanna complex (SAV; 21%) includes a mosaic of grassland with emergent shrubby vegetation and erosion scars. Urban areas (URB; 27%) include industrial, commercial and residential areas; we elected to concentrate our surveys in and around urban residential areas. Together, these six habitat types comprise 95% of Guam's land cover (Figure 3.2).

Sampling methods – We employed standardized visual survey methods to sample snake populations at the 18 sites. Visual surveys provide samples with less bias in size distributions than other methods such as trapping (Rodda et al., 2007). Surveys commenced at sunset and were conducted for three to four hours, including much of the peak activity period of B. irregularis (Rodda et al., 1999a; Lardner et al., 2014). Trained searchers were equipped with powerful headlamps (Lupine Lighting Systems, Dresden, Germany) with desirable beam characteristics that optimize snake detection (Lardner et al., 2009b). Searchers followed habitat edges at a slow pace, roughly 0.5 km per hour, examining visible vegetation and non-vegetative structure for presence of snakes. Forest habitats were surveyed from road edges or footpaths. Savanna searches included road edges, footpaths, and trackless searches throughout the habitat mosaic, including edges of erosion scars. Urban searches were conducted by searching structures and vegetation in residential yards, with all yards separated from large forest tracts by at least one paved road. I selected a target sample size of 100 snakes from each of the 18 sites. To assess seasonal differences in diets, I balanced sample sizes between the wet season (June through November) and dry season (December through May), with at least two quarterly sampling bouts per season. The one exception to this sampling scheme was the second limestone forest replicate (LIM2), which we sampled in one relatively continuous effort due to impending construction; at this site, we collected 90 snakes in the wet season and 10 in the dry season.

Upon visual detection, we hand-captured snakes. Observers recorded time and location of capture, microhabitat data, and morphometric data including snout-vent length (SVL) obtained by stretching the snake along a flexible tape ruler, and weight using Pesola spring scales (Pesola AG, Baar, Switzerland). We transported snakes to the lab on the following morning where SVL and weight were re-measured to validate field data. We euthanized snakes in accordance with approved protocols and performed necropsies. Sex was determined by examination of internal reproductive anatomy. All animal use was conducted in accordance with Colorado State University IACUC Protocol #09-1436A.

Diet composition. We identified prey items in stomach contents to lowest taxonomic order possible given the state of digestion. I tabulated numbers and proportions of prey types by habitat type and totaled them. I recorded items that occurred in aggregate (e.g., clutches of eggs) as single prey takes (e.g., five bird eggs of the same type in one snake were recorded as a single "avian egg" feeding observation). I counted lizard eggs found in association with lizard remains as one lizard prey item, or otherwise recorded them as "lizard egg." I included items only broadly classifiable (e.g., "frog" or "skink") in samples when species-level taxonomic resolution was not required. I compared the distribution of body sizes of snakes containing multiple prey items to that of all snakes containing prey with a two-sample, two-tailed Kolmogorov-Smirnov test and the sex ratio of snakes with multiple prey was compared to that of snakes with single prey with a Fisher's exact test. Where possible, I made direct comparisons to Savidge (1988), the only previous comprehensive diet analysis of Brown Treesnakes on Guam, which was based on collections made between 1982 and 1986 (though including some older records). I also made comparisons with the native-range samples of Greene (1989), Shine (1991a), and Trembath and Fearn (2008) via bar plot and using Fisher's exact test for all statistical tests of equality of proportions.

Variation within and among habitat types – I depicted prey proportions by survey location and pooled by habitat type in proportional stacked bar charts, and used Fisher's exact tests for statistical comparisons of proportions. I investigated similarities in diet composition among all 18 sites by hierarchical clustering (average method based on a correlation distance matrix) using the R package 'pvclust' (Suzuki and Shimodaira, 2013), which generates a clustering dendrogram with approximately unbiased P-values for clusters via multiscale parametric bootstrap resampling. I visually assigned cluster nodes and plotted them on a map of Guam to graphically assess relative geographic aggregation or non-aggregation of sites within clusters. Diet composition for clustering was comprised of the proportion of identifiable prey items of a given taxonomic group to overall number of prey items at a given site. Items not identifiable to species were not considered, with exception of grouping of

all identified or unidentified rodents into a single rodent class. To test seasonal variation in prey composition, I pooled prey items into six major groups (mammals, birds and bird eggs, frogs, geckos, skinks, and other lizards) and I compared wet season samples to dry season samples using Fisher's exact tests.

Factors associated with recent feeding success – I sought to identify individual and ecological factors associated with recent feeding success by conducting logistic regression on presence or absence of prey in stomach contents. Individual factors tested included snout-vent length (*svl*) and its quadratic transformation (*svl*^{2}), *sex*, and body condition index (*ci*). I defined body condition index as an individual's standardized residual from a fourth-order polynomial fit of log (snake mass – prey mass) to log (SVL) for the whole population of snakes collected in this study. I also considered an interaction between sex and SVL (*sex* * *svl*) to allow the relationship between SVL and presence of prey to vary by sex. The ecological factors I tested included site- or habitat-level variation and season (*seas*; wet or dry season). My hypotheses about site- or habitat-level variation were represented by alternative habitat classification schema: the covariate *site* comprises a categorical variable with a level representing each of the 18 sites sampled; *hab* is our nominate habitat classification system, with 6 levels (limestone, scrub, ravine, *Leucaena*, savanna, and urban); *for* lumps all forest types (limestone, scrub, ravine, and *Leucaena*) into a single class and leaves savanna and urban samples to vary independently (3 levels – forest, savanna, and urban); *frsu* is the same as *for*, but allows ravine forests to vary from other forest types due to *apriori* observations of lower snake capture rates and body conditions in ravine forest habitats (4 levels); and, finally, a forest/non-forest (*fnf*) classification that lumps all forest sites into a single class and savanna and urban sites into another non-forest class (2 levels). A season-by-habitat classification interaction term (*seas* * [*class*]) tested for seasonality in prevalence of prey in stomach contents by habitat type. My fully-specified, highest-order model was:

$$logit(p) = \beta_{int} + \beta_{svl} + \beta_{svl^2} + \beta_{ci} + \beta_{sex} + \beta_{sex*svl} + \beta_{seas} + \beta_{[class]} + \beta_{seas*[class]} \qquad (1)$$

where p represented the estimated probability of presence of prey in stomach contents, β_{int} was the regression intercept term, and [class] was one of the habitat classification schemes considered (*site*, *hab*, *for*, *frsu*, or *fnf*). I tested this model against all possible nested models, and based model selection on an information theory approach (Akaike Information Criteria corrected for small sample size, AICc).

Ontogenetic changes in diet – To demonstrate the relationships between snake size and frequency of prey types in stomach contents, I performed logistic regressions on each of seven prey categories (mammals, birds and eggs, frogs, geckos, *Anolis carolinensis*, and two skink species, *Emoia caeruleocauda* and *Carlia ailanpalai*) by major habitat class (forest, savanna, urban), with *svl* and *svl*^{2} terms if significant. I plotted proportions of items of a particular prey class by snake SVL when effects were significant, while proportions of prey items not showing an ontogenetic shift in frequency were included in bar plots. I performed similar logistic regressions by pooling prey into ectothermic (lizard and frog) and endothermic (bird and mammal) classes, and habitats into forest and non-forest types. I represented the relationship between snake size and mass of prey items in a scatterplot of log (prey mass) by SVL. Prey items in advanced states of digestion were not included in this plot. Because preliminary analyses indicated a delayed shift to endothermic prey in forest habitats, I also investigated differences in body condition between mature snakes (>1025 and 1030 mm SVL for females and males, respectively, per the size criteria of Savidge et al., 2007) in forest and non-forest habitats via an ANOVA.

Changes in foraging position associated with body size and prey distributions – I used three lines of evidence to investigate a shift in microhabitat use (arboreal versus terrestrial foraging position). First, to investigate the significance of differences among size distributions

of snakes having preyed on the four most numerous prey species (the skinks *C. ailanpalai* and *E. caeruleocauda* and the geckos *Lepidodactylus lugubris* and *Hemidactylus frenatus*), I performed a quantile regression with snake SVL as the response variable and prey species as a categorical predictor:

$$Q(\tau_{0.05-0.95}|SVL) = \beta_0 + \beta_{[prey]} \tag{2}$$

where Q was the estimate of the τth quantile of SVL for the full range of quantiles from the 0.05^{th} to the 0.95^{th}, and $[prey]$ is a categorical covariate representing one of the four prey species. I coded model contrasts such that quantile means were an average of the quantile estimates for all prey types. I obtained quantile estimates and standard errors (SE) with the linear quantile regression function 'rq()' from 'quantreg' package version 5.05 (Koenker et al., 2013) for R version 3.0.2 (R Core Team, 2013), with 95% confidence intervals estimated at $Q \pm 1.96*$SE. Second, I represented the vertical microhabitat use of these four lizard species in a violin plot based upon 180 field observations of the perch height of each of these species, collected at an intensively-sampled limestone and *Leucaena* forest site in northern Guam since 2005 (U.S. Geological Survey, unpub. data). I obtained average masses for these four lizard species from records of field captures within the Mariana Islands (G. H. Rodda, unpub. data). Lastly, I used a logistic regression to describe the estimated probability, $\hat{p}$, of a Brown Treesnake being found foraging either arboreally or terrestrially as a function of ontogenetic development (*svl*) and broad habitat type (*hab*: forest, savanna or urban) based on the full sample of snakes collected for this study, regardless of presence of prey in stomach contents:

$$logit(p) = \beta_{int} + \beta_{svl} + \beta_{[hab]} \tag{3}$$

Effect of prey distributions on habitat use by large snakes – I also considered three lines of evidence to demonstrate differences in body sizes of snakes among habitat types as influenced by the availability of size-appropriate prey. I performed another quantile regression with

snake SVL as the response variable and the categorical predictor for prey type, [*prey*], re-defined as mammal, bird, frog, skink or gecko. Secondly, I constructed a stacked bar plot depicting the differences in frequencies of ingested endothermic prey (birds and mammals) among habitat types. Finally, I performed another quantile regression with SVL as the response variable and habitat type (forest, savanna, or urban) as the sole predictor:

$$Q(\tau_{0.025-0.975}|SVL) = \beta_{int} + \beta_{[hab]} \tag{4}$$

This regression included all sampled snakes, regardless of the presence of prey in their stomach contents.

Results

Between March 2010 and September 2012, we captured a mean of 100 Brown Treesnakes (n = 99 to 104) at each of the 18 study sites, for a total of 1,805 snakes (990 males, 814 females, and 1 juvenile of undetermined sex). Snakes ranged in size from 331 to 1950 mm SVL, and weighed from 4.7 to 1,727 g. Necropsies were performed on 1,789 snakes (16 very small snakes were not necropsied due to their use in another study). Of these snakes, 555 unique individuals contained 592 prey items, 45 of which were not identifiable to taxon due to advanced digestion. The 547 identifiable prey items were identified to lowest taxonomic order possible (as itemized in Table 1; common names listed in Table 2). Thirty-two snakes (4.1% of total snake sample and 5.8% of snakes containing prey) contained more than one prey item, with four of those containing three prey items. There was no significant difference between size distributions of snakes with single or multiple prey items (P = 0.102) or their sex ratios (P = 0.584). Of prey combinations, 72% were of multiple lizards and 81% were prey from the same taxonomic group. In five cases, a snake containing a frog also contained a lizard, and there was one combination of bird egg and lizard.

Historical and native-range comparisons – Although differences in collection practices may have influenced size distributions of snakes sampled and differences in habitat stratifi-

Table 3.1: Numbers (N) and proportions (%) of prey recovered from Brown Treesnake stomach contents by taxon and habitat type. Non-native species are identified with "*" if introduced in historic times or "†" if presumed to originate from prehistoric introductions.

| | Forested Habitats | | | | | | | | Non-forest | | | | Total | |
| | Limestone | | Scrub | | Ravine | | *Leucaena* | | Savanna | | Urban | | | |
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Birds														
Gallus gallus (domestic)*							1	1.0			7	7.4	**8**	**1.4**
*Passer montanus**											2	2.1	**2**	**0.4**
*Francolinus francolinus**	1	1.1											**1**	**0.2**
Unknown bird									1	1.2	1	1.1	**2**	**0.4**
Bird egg*	1	1.1							3	3.6	4	4.2	**8**	**1.4**
Total birds/eggs	*2*	*2.1*					*1*	*1.0*	*4*	*4.8*	*14*	*14.7*	*21*	*3.8*
Mammals														
*Mus musculus**									2	2.4			**2**	**0.4**
*Suncus murinus**									1	1.2	1	1.1	**2**	**0.4**
Rattus sp.*†	2	2.1	1	0.9					5	6.0			**8**	**1.4**
Unknown rodent*			1	0.9					11	13.1	1	1.1	**13**	**2.4**
Unknown mammal*									3	3.6			**3**	**0.5**
Total mammals	*2*	*2.1*	*2*	*1.8*					*22*	*26.2*	*2*	*2.1*	*28*	*5.1*
Reptiles														
Hemidactylus frenatus	8	8.4	15	13.8	7	10.4	12	12.4	4	4.8	24	25.3	**70**	**12.8**
Lepidodactylus lugubris	17	17.9	9	8.3	6	8.6	19	19.6	12	14.3	3	3.2	**66**	**12.1**
Gehyra mutilata†					1	1.5							**1**	**0.2**
Nactus pelagicus	2	2.1			1	1.5							**3**	**0.5**
Unknown gecko	11	11.6	12	11.0	8	11.9	17	17.5	10	11.9	15	15.8	**73**	**13.3**
*Carlia ailanpalai**	19	20.0	17	15.6	2	3.0	19	19.6	12	14.3	8	8.4	**77**	**14.1**
Emoia caeruleocauda	15	15.8	19	17.4	27	40.3	19	19.6	2	2.4	3	3.2	**85**	**15.5**
Lipinia noctua	1	1.1			1	1.5							**2**	**0.4**
Unknown skink	10	10.5	13	11.9	5	7.5	5	5.2	9	10.7	2	2.1	**44**	**8.0**
*Anolis carolinensis**			1	0.9					6	7.1	20	21.1	**27**	**4.9**
Varanus indicus†	1	1.1	2	1.8	1	1.5	1	1.0					**5**	**0.9**
Unknown lizard											1	1.1	**1**	**0.2**
Unknown lizard egg	4	4.2	2	1.8			4	4.1	2	2.4	1	1.1	**13**	**2.4**
Total reptile/eggs	*88*	*92.6*	*90*	*82.6*	*59*	*88.1*	*96*	*99.0*	*57*	*67.9*	*77*	*81.1*	*467*	*85.4*
Amphibians														
*Eleutherodactylus planirostris**	3	3.2	15	13.8	4	5.6					2	2.1	**24**	**4.4**
*Hylarana guentheri**					2	3.0							**2**	**0.4**
*Polypedates megacephalus**					1	1.5							**1**	**0.2**
Unknown frog*			2	1.8	1	1.5			1	1.2			**4**	**0.7**
Total amphibians	*3*	*3.2*	*17*	*15.6*	*8*	*11.9*			*1*	*1.2*	*2*	*2.1*	*31*	*5.7*
TOTAL	**95**		**109**		**67**		**97**		**84**		**95**		**547**	**100**

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Table 3.2: List of common names for prey species found in stomach contents.

BIRDS

Gallus gallus	Domestic Chicken
Francolinus francolinus	Black Francolin
Passer montanus	Eurasian Tree Sparrow

MAMMALS

Mus musculus	House Mouse
Mus musculus	House Mouse
Rattus sp.	Rat species
Suncus murinus	Asian House Shrew

REPTILES

Hemidactylus frenatus	Mediterranean House Gecko
Lepidodactylus lugubris	Mourning Gecko
Gehyra mutilata	Mutilating Gecko
Nactus pelagicus	Rock Gecko
Carlia ailanpalai	Curious Skink
Emoia caeruleocauda	Blue-tailed Skink
Lipinia noctua	Moth Skink
Anolis carolinensis	Green Anole
Varanus indicus	Mangrove Monitor

AMPHIBIANS

Eleutherodactylus planirostris	Greenhouse Frog
Hylarana guentheri	Günther's Barking Frog
Polypedates megacephalus	Hong Kong Whipping Frog

cation, a comparison of our results with those of Savidge (1988) indicated that the current sample was characterized by a lower proportion of snakes containing prey (31% vs. 52%, P < 0.001), fewer birds and bird eggs (3.8% vs. 28.1%, P < 0.001), fewer mammals (5.1% vs. 8.7%, P < 0.05), more lizards and lizard eggs (85.4% vs. 63.2%, P <0.001) in stomach contents, and frogs comprising 5.7% of stomach contents when no frogs were observed in previous samples (P < 0.001)(Figure 3.3). In comparison, 64 prey items recovered from stomachs of museum specimens from the native range (excluding Australia) by Greene (1989) included 20.3% birds and bird eggs, 46.9% mammals, 29.7% lizards and eggs, and 3.1% frogs. From the New South Wales, Northern Territory, and Queensland regions of Australia, Shine (1991a) recorded prey ratios of 35.8% birds and bird eggs, 23.2% mammals, 34.7% lizards, and 6.3% frogs. The Trembath and Fearn (2008) samples from northern Australia documented 28 prey items comprised of 46.4% birds, 28.6% mammals, 21.4% lizards and 3.6% frogs. Prey proportions were significantly different between the Greene and Shine samples, primarily differing in higher proportions of mammals in the Greene sample and higher proportions of birds in the Shine sample; neither was significantly different than the Trembath & Fearn sample, though with a sample size of only 28 prey items there was not enough statistical power to detect differences. All pairwise comparisons of Guam diets with native range diets and between the two Guam samples indicated highly significant differences in prey compositions (P << 0.001).

Variation within and among habitat types – Diet composition was moderately to significantly variable within and among most habitat types (Figure 3.4). In limestone forest, diets were predominantly geckos and skinks and did not vary among replicates (P = 0.207). Variation within scrub forest was significant (P < 0.001), primarily due to a high proportion of frogs and low proportion of geckos at SCR1. Among ravine forest sites, the RAV2 sample had more geckos than skinks and no frogs, resulting in significant variation among replicates (P = 0.013). Variation was significant within *Leucaena* replicates (P = 0.005) with a lower ratio of geckos to skinks and one avian prey item recorded at LEU1. Savanna
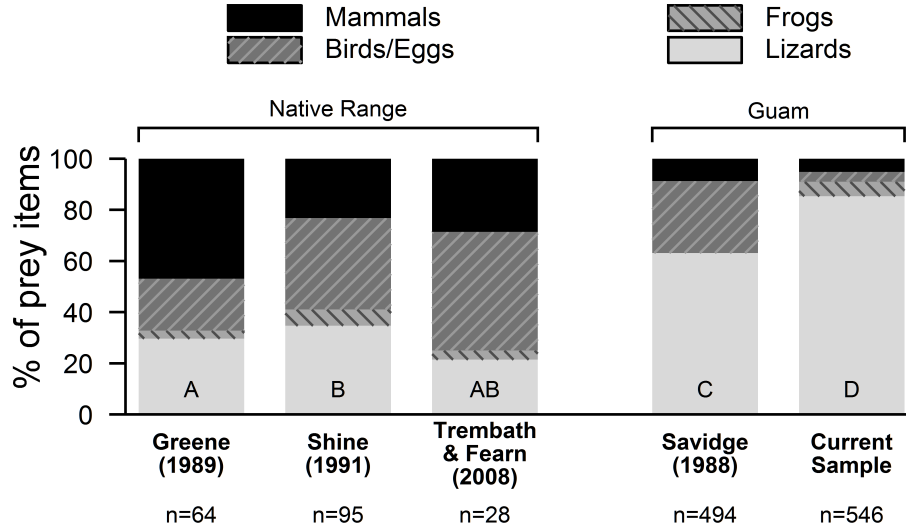
Figure 3.3: Comparisons of proportions of classifiable prey items between non-Australian (Greene, 1989) and Australian (Shine, 1991; Trembath and Fearn, 2008) portions of the native range, the historical collection from 1980-1985* (Savidge, 1988; *note, this sample included records of some snakes from 1967-1981), and the current sample. Letters on bars represent groupings that were statistically distinguishable (Fisher's exact test, $\alpha = 0.05$).

sites varied significantly (P = 0.028), with a higher ratio of geckos at SAV1 and the highest proportion of mammals at SAV3. Urban sites exhibited the most variability (P < 0.001), with almost exclusively geckos in snake diets at URB1, a large proportion of other lizards (all *A. carolinensis*) at URB2, and the only urban frogs at URB3.

When replicates were pooled by their respective habitat types, significant differences existed among the four forest habitats (P < 0.001). Although there were no significant differences in diet composition between limestone, *Leucaena*, and ravine forests or between scrub and ravine forests, overall differences among the four forest habitats were significantly different (P < 0.001) and primarily due to considerable proportions of frogs in scrub and ravine forest diets. Among forest, savanna and urban groupings, dietary differences were obvious with more frogs in forests, more birds and *A. carolinensis* in urban sites, and more mammals in savanna habitat. The overall difference was significant at P < 0.001 and each pairwise comparison among the three habitat classifications was significant at P < 0.001.

Hierarchical clustering by similarity in diet composition among all 18 sites (Figure 3.5)
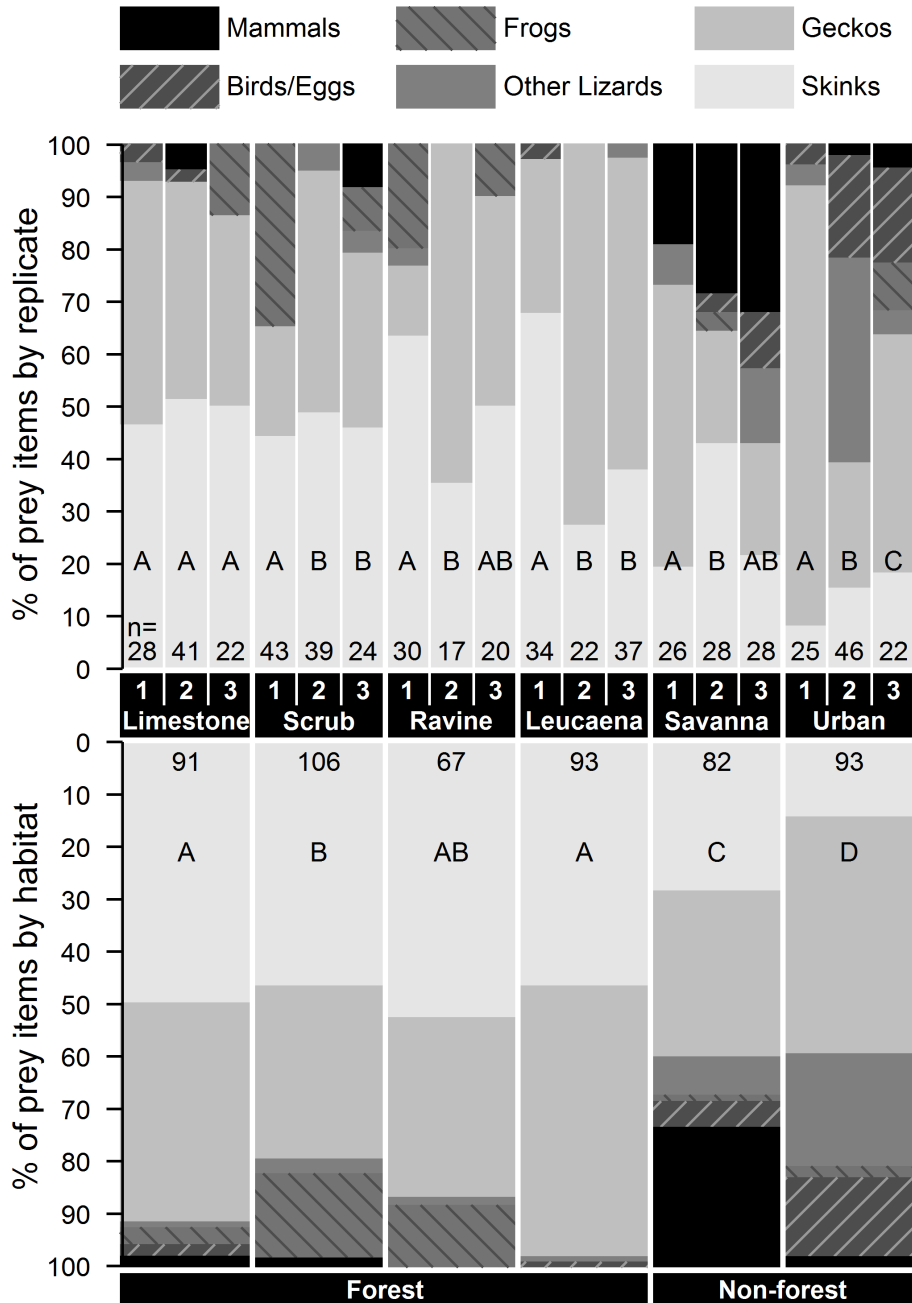
Figure 3.4: Diet composition by replicate (top bars) and by habitat type (bottom bars), including the total number (n) of prey items collected in stomach contents from each replicate or habitat. Letters on bars represent groupings that were statistically distinguishable (Fisher's exact test, $\alpha = 0.05$), with within-habitat comparisons (top bars) restricted to the respective habitat type.

showed considerable similarities among sites within habitat classes, with relatively minor similarities between sites based on geographic location. Clusters A, B, and C contained all urban and savanna sites. All three savanna sites clustered together under the B node, likely due to higher proportions of mammals (grassland rodents) in this habitat. Urban sites tended to be characterized by more avian prey. The C node (URB1 and URB3) clustered more closely to forest sites; these sites were in closer geographic proximity to forest habitat than was the more isolated and urbanized URB2, which was an outlier, being the only site to have Eurasian Tree Sparrows (*Passer montanus*) in stomach contents and the highest prevalence of the introduced lizard *A. carolinensis*, which made up 45% of identifiable prey items from that site. Among forest habitats, SCR1 was a sole outlier with 46.6% of all diet items being introduced Greenhouse Frogs (*Eleutherodactylus planirostris*), far more than at any other site. Two of three limestone forest sites clustered under node E, two of three ravine forest sites clustered under node F, and two of three *Leucaena* sites clustered under G. Geographically, all sites under node F were aggregated in eastern south-central Guam (map in Figure 3.5). However, the remaining clusters based on diet similarity were broadly dispersed throughout Guam. These results suggest more correlation in diet composition within habitat types as opposed to spatial autocorrelation among adjacent sites.

Seasonal variation – Seasonal diet compositions, based on pooling prey into six prey classifications, did not change significantly between wet and dry seasons in any of the four forest habitat types (limestone, P = 0.464; scrub, P = 0.154; ravine, P = 0.269; *Leucaena*, P = 0.321). However, when all forest types were pooled together, diets in forests differed between seasons (P = 0.009), with more frogs and fewer geckos and skinks during the dry season. There was no seasonal difference in savanna diets (P = 0.524), but urban diets were composed of more geckos and fewer *A. carolinensis* in the dry season (P = 0.011).

Factors associated with recent feeding success – Snake size alone was not a significant predictor of presence of prey in stomach contents. However, addition of a quadratic term, svl^2, resulted in a greater than 13 AICc unit improvement over the null model. Together,

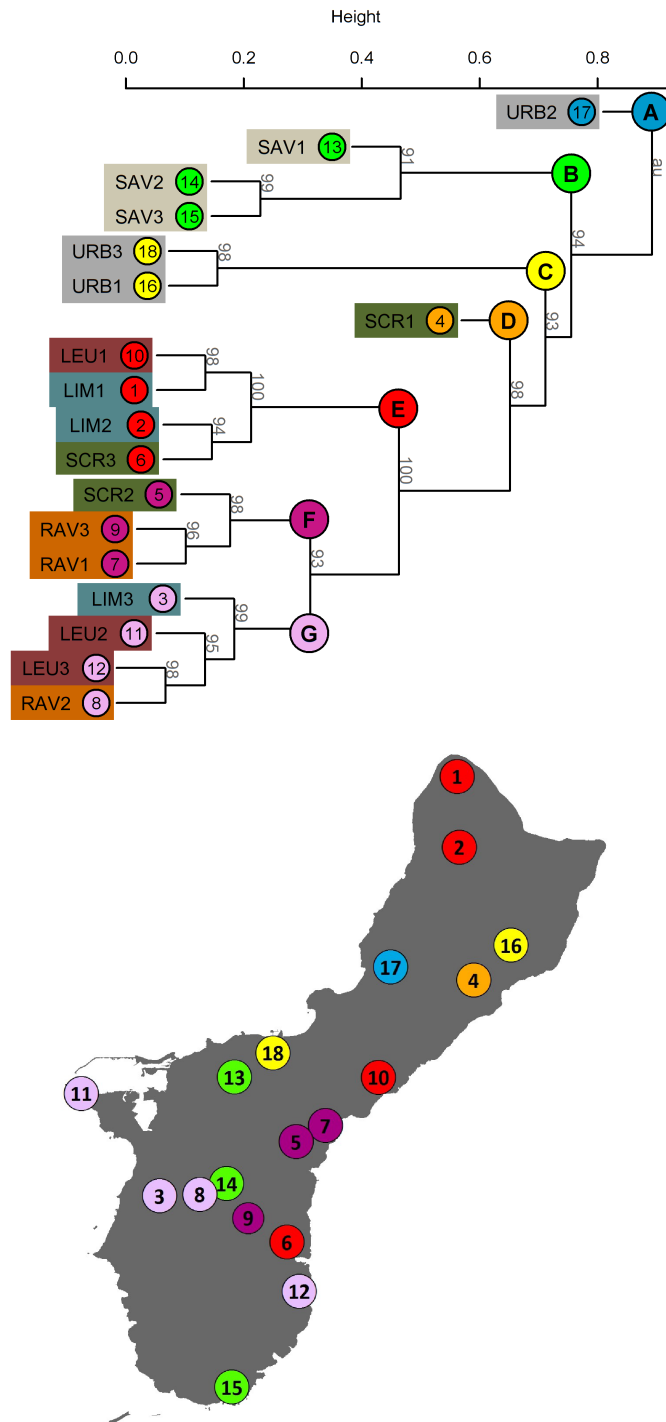Figure 3.5: Dendrogram representing similarities in species-level diet composition among all 18 surveyed sites, with corresponding locations indicated on the map. Numerical values on dendrogram edges denote approximately unbiased P-values, with high values (e.g., 95%) strongly supported. "LIM" = limestone forest, "SCR" = scrub forest, "RAV" = ravine forest, "LEU" = *Leucaena* forest, "SAV" = savanna, and "URB" = urban.
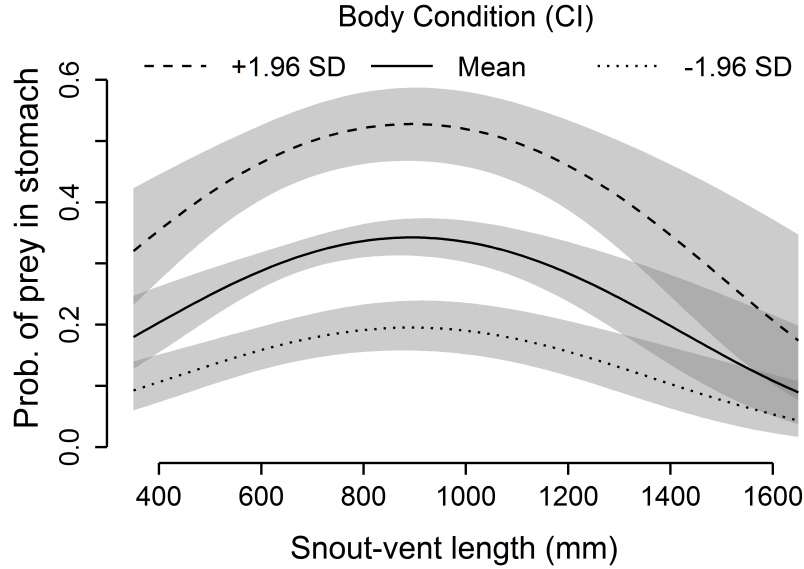
Figure 3.6: Effects of snout-vent length ($svl+svl^2$) and body condition (ci) on the probability of finding prey in stomach contents of Brown Treesnakes. Gray polygons represent the 95% confidence intervals for the estimates. In addition to the mean body condition value, the values of $+1.96$ and -1.96 are in units of standard deviation, i.e., 95% of observed body conditions fall between these two values.

svl and svl^2 were included in all models carrying 100% of Akaike model weights. Parameter estimates for the quadratic term indicted an inverted U-shaped influence of SVL, with prevalence of prey in stomach contents significantly lower in smaller and larger snakes, and highest in snakes ~ 800 to 1000 mm SVL (Figure 3.6). Body condition (ci) was also a highly influential factor with parameter estimates indicating a higher probability of recent feeding success for snakes in better body condition. The effect of sex indicated a slightly higher prevalence of prey in stomachs of female snakes, though predicted confidence intervals for male and female estimates overlapped broadly. Season ($seas$) was included in models carrying 87% of Akaike weights, with probability of prey in stomach contents higher during the wet season, though effect size was modest and confidence limits overlapped. Beyond inclusion of these factors, there was little discriminatory power between top models, due primarily to minor differences in including the habitat classification alternatives and the $sex * svl$ interaction term. These results indicate no appreciable effect of site or habitat classification on recent feeding success.

Ontogenetic shifts in diet composition and prey size – Logistic regression on prey frequency by SVL revealed significant ontogenetic shifts in prey proportions from almost exclusively geckos at smaller size classes to mammals and birds at larger size classes, with higher proportions of *C. ailanpalai* at intermediate size classes in forest and savanna habitats (Figure 3.7A&B). Within forest habitats (Figure 3.7A), *C. ailanpalai* appeared to be an important prey source for intermediate and large snakes, with mammals increasing in importance with snake size. There was no significant effect of SVL on bird, frog, *A. carolinensis*, and *E. caeruleocauda* proportions. Within savanna habitats (Figure 3.7B), proportions of mammals and birds increased significantly with SVL. *C. ailanpalai* were a relatively low proportion of stomach contents in savannas, with proportions peaking at much smaller sizes (∼850 mm) when compared to forest snakes (∼1250 mm). Proportions of frogs, *A. carolinensis*, and *E. caeruleocauda* were not significantly related to snake size. Proportions of mammals and birds were significantly related to snake size in urban habitats (Figure 3.7C), with birds being the most prevalent prey for larger snakes. Proportions of frogs, *A. carolinensis*, *C. ailanpalai*, and *E. caeruleocauda* were not related to snake size within urban habitats.

Comparing proportions of ectothermic and endothermic prey by snake size in forest versus non-forest habitat types, the size at which a non-forest snake was just as likely to contain an ectothermic or endothermic prey (proportion = 0.50) was 1025 mm SVL, while that probability was not achieved in a forest snake until the size of 1475 mm (Figure 3.8). This indicates a significant delay in the size at which forest snakes are able to obtain the prey that fulfill the feeding preferences associated with their ontogenetic prey shift. Corresponding with the lack of large prey, mean body condition for adult-sized snakes in forest habitats was significantly lower than that for snakes in urban and savanna habitats where birds and mammals were consumed more frequently and by smaller snakes ($P < 0.001$; Figure 3.9).

As is common for many snakes (Shine, 1991b), Brown Treesnakes in our sample exhibited a true ontogenetic shift in prey size, where larger snakes dropped smaller prey items from their diet as evidenced by the lack of points in the lower right triangle of Figure 3.10, as
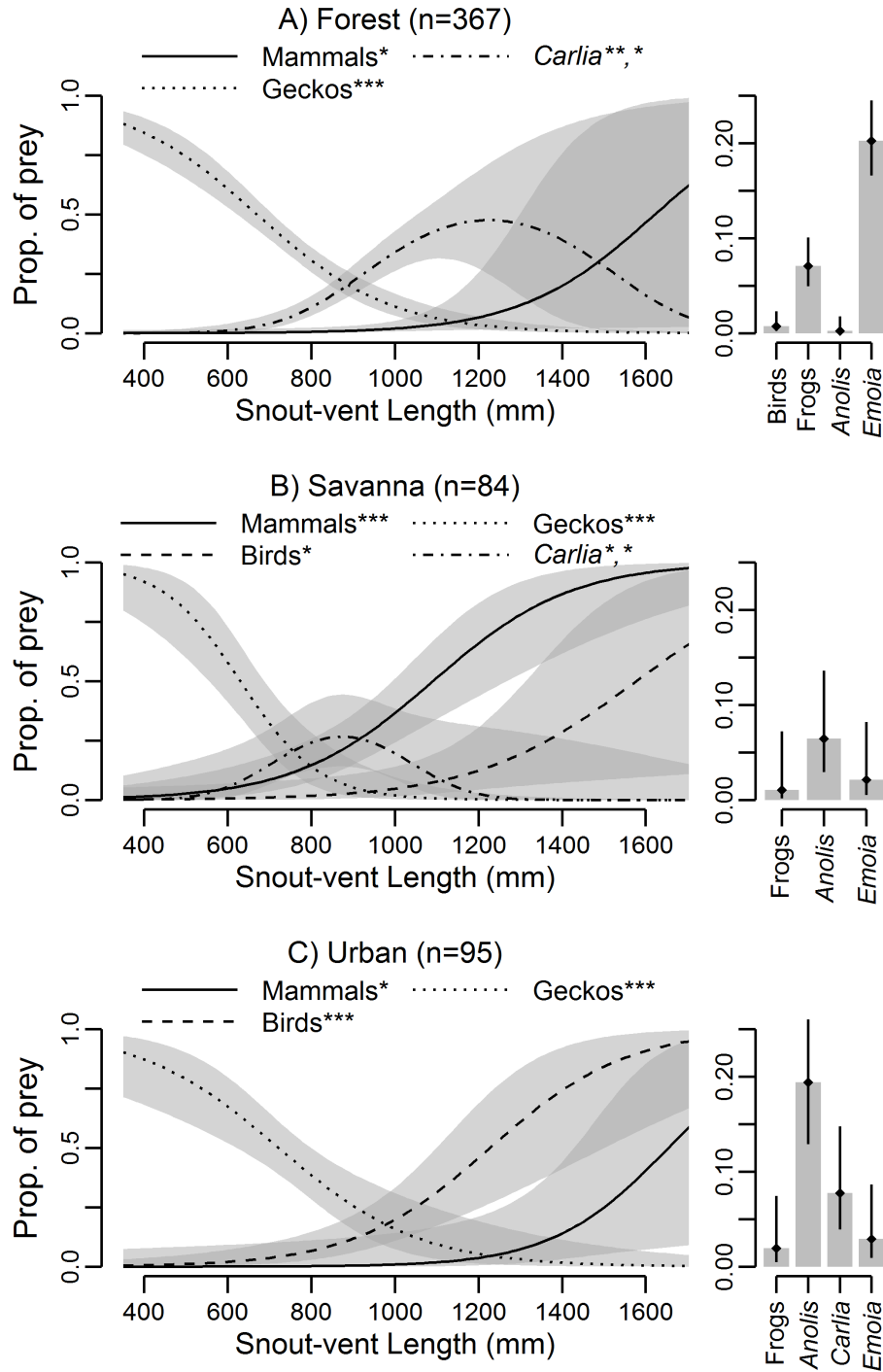
Figure 3.7: Proportions of prey items in diets as predicted by logistic regressions of each prey type by habitat. Proportions and 95% confidence polygons are plotted against snake snout-vent length (SVL) to demonstrate ontogenetic shifts where svl or svl^2 terms were significant, or as bars with 95% confidence limits (right panes) where they were not. "*" = P < 0.05, "**" = P < 0.0l, "***" = P < 0.00l. An asterisk after a comma indicates the significance of the svl^2 quadratic term. "$Carlia$" = C. ailanpalai, "$Emoia$" = E. caeruleocauda, "$Anolis$" = A. carolinensis.
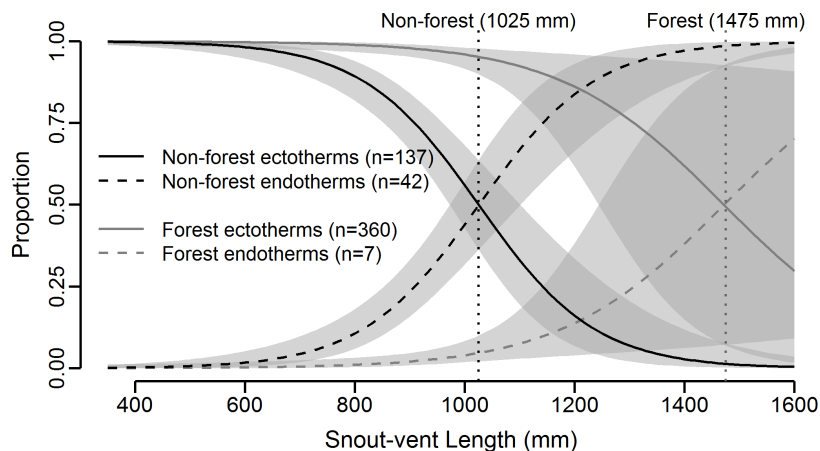
Figure 3.8: Logistic regression results depicting the ontogenetic transition from ectothermic prey (lizards and frogs) to endothermic prey (birds and mammals) by snakes in forest and non-forest habitat types. The vertical dashed lines indicate the size at which the probability of a prey item being ectothermic or endothermic was equal (50%). Note the very broad confidence intervals for the larger SVLs in forest habitat resulting from a small sample size in this range.
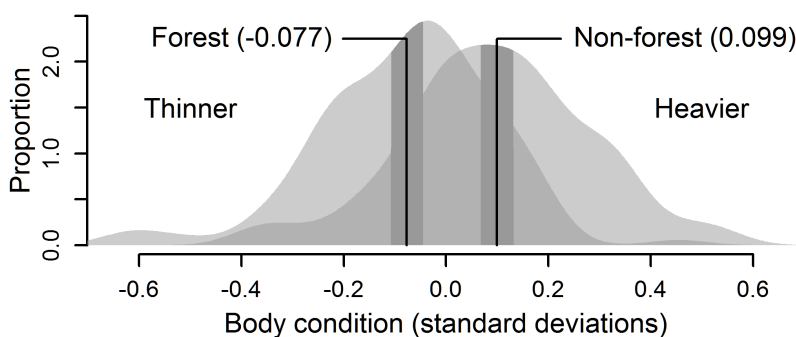
Figure 3.9: Distribution of body condition (residual body mass) estimates in units of standard deviations, with estimated means for adult snakes (females >1025 mm and males >1030 mm SVL, per Savidge et al. 2007) in forest versus non-forest samples. Darker gray zones represent the 95% confidence intervals for the estimates ($\pm$ 1.96*SE).

Figure 3.10: Relationship between prey mass and snake size. Polygons contain all of the observed values for the prey types that showed significant ontogenetic shifts in frequency (mammals, birds, geckos and *Carlia*). The lack of points in the lower right triangle indicate an ontogenetic shift in the lower size limit of prey. "*Carlia*" = *C. ailanpalai*, "*Emoia*" = *E. caeruleocauda*, "*Anolis*" = *A. carolinensis*.

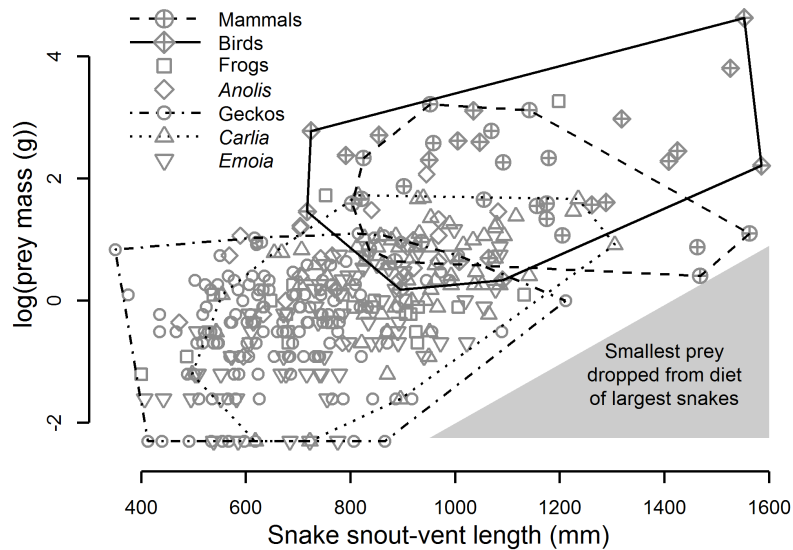opposed to an "ontogenetic telescope" (Arnold, 1993) where larger snakes take larger prey and continue to take smaller prey as well.

Changes in foraging position – The results of the quantile regression on SVL for the snakes that contained identifiable lizard prey of the four predominant species (Figure 3.11A) indicate that snakes taking the invasive skink *C. ailanpalai* were significantly larger throughout the entire quantile range than the quantile mean of all snakes containing prey. Snakes taking the native skink *E. caeruleocauda* were of relatively average size throughout the quantile range. The geckos *H. frenatus* and *L. lugubris* were taken by significantly smaller snakes, with no statistical difference in SVL quantile estimates between them. Field observations of the vertical position of these four lizard species (Figure 3.11B) indicate that *C. ailanpalai* are almost exclusively terrestrial, *E. caeruleocauda* are observed terrestrially and to a smaller degree arboreally, and the two gecko species are almost exclusively arboreal. The results of the logistic regression on whether all snakes collected in this study were found foraging arboreally or terrestrially (Figure 3.11C) indicate a strong and highly significant shift from

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almost exclusive arboreal foraging for smaller size classes to almost exclusively terrestrial foraging for larger snakes in forest habitat (less so in savanna and particularly urban habitats). Taken together, the three comparisons in Figure 3.11 indicate that Brown Treesnakes of different size classes differ in microhabitat use (vertical foraging position) in response to differences in the vertical distribution of size-appropriate prey resources.

Field records for masses for these four species further support a relationship between prey size and snake size. The terrestrial *C. ailanpalai*, taken by the larger snakes, had the greatest average mass (2.66 g, SE ± 0.034, n = 2041). *L. lugubris*, taken by the smallest snakes, had the lowest average mass (1.07 g, SE ± 0.020, n = 887). *H. frenatus*, taken by gecko-eating snakes slightly larger than those taking *L. lugubris*, had a higher mass (2.23 g, SE ± 0.035, n = 691). *E. caeruleocauda*, taken by relatively average-sized snakes, were intermediate in mass between *C. ailanpalai* and *L. lugubris* (1.69 g, SE ± 0.020, n = 1410).

Effect of prey distributions on habitat use by large snakes – Quantile regression of Brown Treesnake SVL by prey type (Figure 3.12A) demonstrates that the size distributions of snakes taking bird and mammal prey were significantly larger than the distributions of snakes taking skinks and frogs as prey, while the size distribution of snakes taking geckos is significantly lower across the entire quantile range. Mammals and birds were very rarely observed in the stomach contents of forest snakes (Figure 3.12B) compared to the much higher abundances of mammals and birds in savanna and urban habitats. Although small and medium-sized snakes were found in all habitats, the largest snakes in savanna and urban habitats were significantly larger than the largest snakes in forest habitats (Figure 3.12C).

Discussion

Brown Treesnakes are adaptable generalist predators, taking nearly all vertebrate prey of manageable size. Employing visual and olfactory cues (Chiszar, 1990) and a combination of sit-and-wait and active predation (Rodda, 1992), they forage nocturnally for active (e.g., geckos) and inactive prey (e.g., eggs, diurnal birds, and skinks). We considered stomach
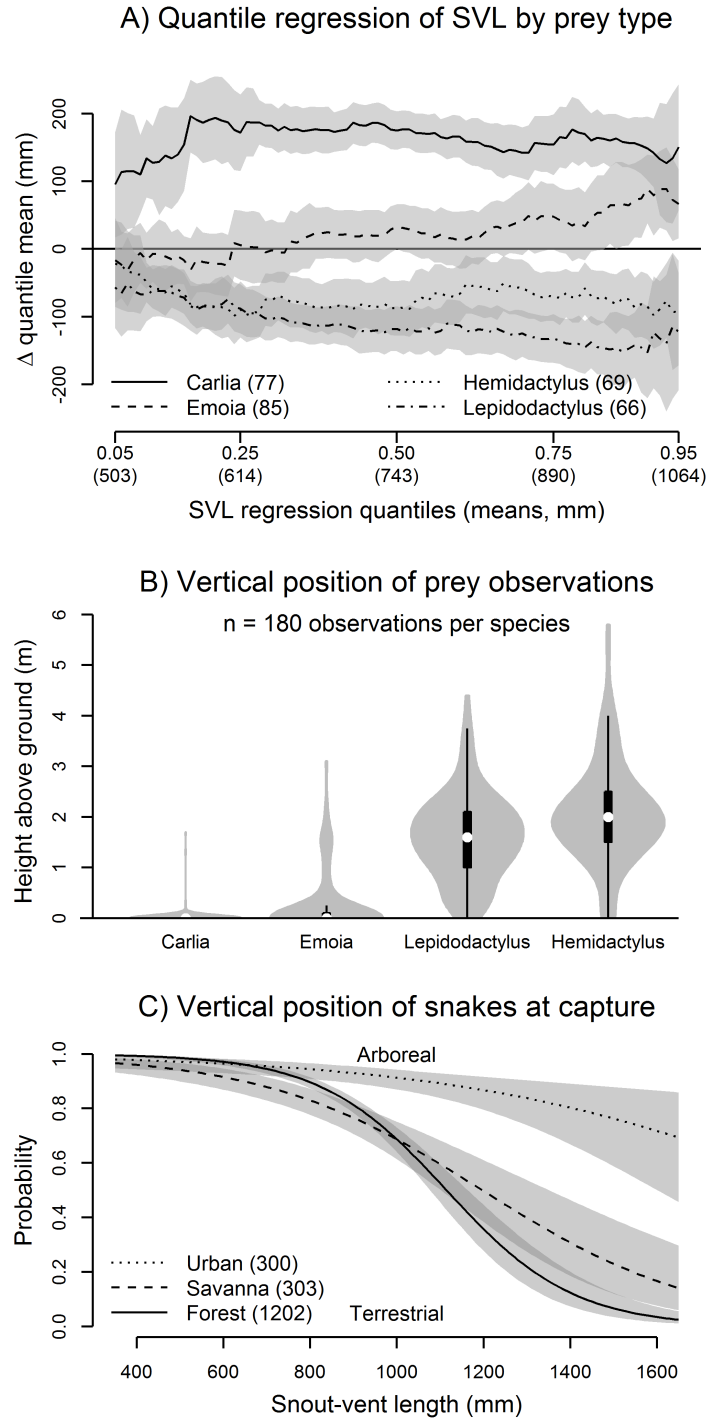
Figure 3.11: Evidence for a vertical foraging shift during the ontogeny of Brown Treesnakes on Guam. A) Quantile SVL means throughout the entire quantile range for snakes containing identifiable small lizard prey; the zero-line is the quantile mean for all snakes containing prey, and non-overlapping of 95% confidence interval polygons indicates a significant difference. B) Distributions of height above ground for observations of each species. C) Logistic regression estimates for probability of snakes being found foraging arboreally versus terrestrially by SVL and major habitat type. "*Carlia*" = *C. ailanpalai*, "*Emoia*" = *E. caeruleocauda*, "*Lepidodactylus*" = *L. lugubris*, "*Hemidactylus*" = *H. frenatus*.

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A) Quantile regression of SVL by prey type

Legend:
- Mammals (28) (dashed)
- Birds (21) (solid)
- Frogs (31) (dotted)
- Skinks (208) (gray solid)
- Geckos (209) (gray dashed)

Y-axis: Δ quantile mean (mm)

X-axis: SVL regression quantiles (means, mm)
0.05 (515), 0.25 (653), 0.50 (784), 0.75 (923), 0.95 (1124)

B) Endothermic prey by habitat

Legend: Bird (gray), Mammal (black)

Y-axis: Proportion of prey

X-axis: Habitat type
Forest (n=357), Savanna (n=82), Urban (n=93)

C) Quantile regression of SVL by habitat

Legend:
- Forest (1202) (dotted)
- Savanna (303) (dashed)
- Urban (300) (solid)

Y-axis: Δ quantile mean (mm)

X-axis: SVL regression quantiles (means, mm)
0.025 (433), 0.25 (623), 0.50 (765), 0.75 (923), 0.975 (1377)

Figure 3.12: Evidence that the presence of larger snakes in savanna and urban habitats was correlated with prevalence of bird and mammal prey in stomach contents. A) Quantile SVL means throughout the entire quantile range for snakes containing mammal, bird, frog, skink or gecko prey, with the zero-line at the quantile mean for all snakes containing prey and non-overlapping of 95% confidence interval polygons indicating significant differences. B) Proportions of birds and mammals in stomach contents of snakes by habitat. C) Quantile regression results indicating SVL quantile means of all snakes by habitat type.

contents of all snakes at one site to be a reflection of the snake's realized foraging niche at that location and compared that sample to prey in stomach contents at other sites. With the exception of limestone forest replicates, in which Brown Treesnake diets did not differ, in all other cases we found moderate to considerable variability in proportions of prey taken among the replicates within a habitat type and among the various habitats. As might be considered logical, similarities in diet compositions seem to be greater among sites within habitat types than between neighboring sites (Figure 3.5) which suggests that ecological influences on prey availability are greater than any regional influences or spatial autocorrelation in diet composition.

The probability of a snake having had recent foraging success, as evidenced by presence of prey in stomach contents, was highly influenced by snake size and body condition (Figure 3.6). At least two non-exclusive alternative hypotheses may explain the peak of predicted probability in middle size ranges. First, very small snakes may be less successful at capturing prey, and the size of an average small lizard relative to the size of a small snake may result in a longer period of satiation, while larger snakes may encounter bird and mammal prey more infrequently due to their scarcity, and undergo a similar prolonged satiation after capturing such large prey; hence, snakes having taken large prey with respect to their body size may exhibit decreased detectability. If such a satiation effect on detectability exists, this would have meaningful implications for management of invasive populations, particularly in environments rich in large prey. Alternatively, although very small snakes appear to be limited to gecko prey and large snakes to birds and mammals, snakes of median size classes can capitalize on all prey types (Savidge, 1988; Figure 3.7). The strong positive effect of body condition suggests that snakes in good condition are likely to obtain prey more frequently. Neither sex nor season appears to have a significant effect on the probability of an individual containing prey. The lack of site-by-site variability or habitat effect demonstrates that snakes in all habitats, after controlling for size and body condition, are equally likely to have prey in stomach contents. This result conforms to predictions of an ideal free distribution (Fretwell

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and Lucas, 1970), in that densities of foraging snakes appear to be in equilibrium with prey densities regardless of location or habitat, at least as reflected by feeding frequency.

With respect to differences in food resource use among size classes of Brown Treesnakes, our results confirm a strong and consistent ontogenetic prey shift as is common for many snake species (Mushinsky, 1987; Shine, 1991b) and has been documented in Brown Treesnakes and their close relatives (Savidge, 1988; Greene, 1989). The shift from ectothermic to endothermic prey appears to go beyond the mechanical considerations of allometry and prey size and is apparently an intrinsic maturational change (e.g., Mushinsky and Lotz, 1980). Juveniles in feeding trials almost exclusively forgo skinks and neonate mice for geckos of the same size, with the smallest snakes showing the strongest preference (Lardner et al., 2009a), and large captive snakes have been anecdotally reported to refuse lizard prey, even when not recently fed. Additionally, the venom of juvenile Brown Treesnakes is more toxic to lizards, with taxa-specific toxicity shifting toward birds as snakes mature (Mackessy et al., 2006).

Vertical habitat shifts have been observed in several lizard and snake species (e.g., Stamps, 1983; Shine and Li-Xin, 2002; Imansyah et al., 2008). Increased terrestrial foraging by larger Brown Treesnakes on Guam (Figure 3.11) reflects an adaptive behavioral shift in response to differences in the vertical distributions of prey of varying size. Arboreally-foraging small snakes subsist almost entirely on small arboreal lizards (Figure 3.7). Larger snakes would normally shift to a diet higher in bird content as evidenced by taxa-specific toxicity venom of adult snakes and feeding records from the native range and Guam urban habitats where bird prey persist. As most forest birds normally roost and nest arboreally, it is logical that larger snakes under normal ecological conditions would also forage arboreally. This reasoning is supported by the higher proportion of arboreally-foraging large snakes collected within relatively bird-rich urban habitats (Figure 3.11C). Within forest habitats, the shift to terrestrial foraging by larger snakes appears to be a behavioral response to the lack of larger arboreal prey and the availability of larger terrestrial lizards (non-native *C. ailanpalai*). We have little doubt that larger Brown Treesnakes in Guam's forests would resume more frequent

arboreal foraging if roosting birds re-established in these habitats.

We found a higher incidence of larger snakes in savanna and urban habitats, where rodent and bird prey persist (Figure 3.12). Without long-term tracking of individual snakes throughout the maturational process, we cannot discern if larger individual snakes search for and actively select savanna or urban habitats. It is also plausible that larger snakes within forest habitats either fail to grow into the larger size classes or experience increased mortality due to a lack of size-appropriate prey resources; a lower average body condition for mature snakes in forest versus non-forest habitats (Figure 3.9) lends weight to this argument as snakes in lower body condition may grow in length at a slower rate. In all likelihood, because Brown Treesnakes are highly mobile active foragers without defined activity areas (Santana-Bendix, 1994), the true nature of this apparent shift in habitat use will lie along a continuum from active habitat selection to differential growth and mortality. The pattern of larger Brown Treesnakes being found in urban habitats was previously observed as far back as the 1980s by Savidge (1991), shortly after the collapse of bird and mammal prey in forest habitats. As with the observed vertical habitat shift by larger snakes, this pattern in size-biased habitat use would probably diminish or disappear if abundant bird and mammal prey were to re-establish in Guam's forest habitats. An increased rate of feeding on large introduced frogs (e.g., *Fejervarya* or *Hylarana* spp.) might also result in greater use of forest habitats by larger snakes where these frogs are abundant.

Current Brown Treesnake populations on Guam are subsisting on a diet radically different from that in their home range (Figure 3.3), specifically in very low proportions of bird and mammal prey, and particularly in forest habitats (Figure 3.4). However, sufficient prey resources persist to allow them to maintain abundances far higher than most "natural" snake populations (Rodda et al., 1999a). Based on sampled or observed abundances of prey, variations in the diet of this generalist vertebrate predator appear to reflect variation in prey communities. However, Brown Treesnakes are not perfectly non-biased samplers of prey, as some species are taken out of proportion to our perceptions of their abundance.

For example, although observers have recorded thousands of frog sightings during our visual surveys, frogs remain a small portion of Brown Treesnake diets. Because prey choice also varies by size class of snake (Figure 3.7; Savidge, 1988; Greene, 1989; Lardner et al., 2009a), relative importance of different prey may vary geographically as a result heterogeneity in snake size distributions (Chapter One), with possible management consequences (Chapter Two). We discuss the importance of various prey as they differ by location, habitat type, and snake size class:

Birds – Birds may be the naturally preferred prey type for mature Brown Treesnakes (Greene, 1989; Mackessy et al., 2006; Trembath and Fearn, 2008). However, in Guam, birds only persist as a significant dietary item in urban habitats, and these birds are all non-native domestic (e.g., Domestic Fowl, *Gallus gallus*) or commensal (e.g., *P. montanus*) species. In forest habitats, only one bird item, a wing of a single specimen of Black Francolin, *Francolinus francolinus* – an introduced gamebird – was found in stomach contents. This low predation on birds in forests is certainly due to a lack of available bird prey resulting from Brown Treesnake predation (Savidge, 1987; Wiles et al., 2003).

Mammals – On Guam, Brown Treesnakes prey on small mammals at a rate far lower than recorded from their native range. Rodent and shrew densities and biomasses are about an order of magnitude lower on Guam than on neighboring snake-free islands, particularly in forest habitats (Wiewel et al., 2009). While still suppressed in numbers, they remain somewhat abundant only in savanna grassland habitats, where their populations may have adequate resources and refuge to persist under snake predation. All small mammals we found in Brown Treesnake diets are introduced species that may have undesirable food web and human nuisance impacts. Guam's only remaining native mammal is the endangered Mariana Fruit Bat (Pteropus mariannus) whose Guam population has declined to critical levels. In addition to illegal hunting by humans, predation of young bats by *B. irregularis* may hamper recovery of this population (Wiles, 1987; Rodda et al., 1997).

Reptiles – Lizards dominated the diets of Brown Treesnakes in all habitats, with skinks

and geckos comprising 82.6% to 99.0% of diet items in forests (Table 1). Aside from uncommon takes of relatively rare Mutilating Geckos, *Gehyra mutilata*, and Rock Geckos, *Nactus pelagicus*, gecko prey are almost exclusively either *H. frenatus* or *L. lugubris*. Both species are arboreal and nocturnal, and have been present on Guam since prehistoric times (Pregill, 1998). Although they occur in stomach contents in nearly equal proportions, density estimates consistently demonstrate that *H. frenatus* are as much as twice as abundant as *L. lugubris* (Rodda and Fritts, 1992; Campbell, III et al., 2012; G. H. Rodda, unpub. data). This apparent electivity may be due to the average mass of a *L. lugubris* being approximately half that of *H. frenatus* (G. H. Rodda, unpub. data), making them an acceptable size for a wider range of snakes. Alternatively, *H. frenatus* may have differences in habitat use or predator avoidance abilities that result in the species being taken less frequently than in proportion to their abundance. Aside from two rare Moth Skinks, *Lipinia noctua*, all identifiable skinks taken by Brown Treesnakes were either invasive *C. ailanpalai* or native *E. caeruleocauda*. Adult *C. ailanpalai* are the largest of the lizards commonly taken by Brown Treesnakes and are almost exclusively terrestrial. These two skinks show a similar pattern of consumption by Brown Treesnakes; although abundance estimates indicate that *C. ailanpalai* are typically more numerous than *E. caeruleocauda* (Rodda and Fritts, 1992; Campbell, III et al., 2012; G. H. Rodda, unpub. data), *E. caeruleocauda* are found in stomach contents at a slightly higher frequency than *C. ailanpalai*. In addition to *E. caeruleocauda* being smaller than *C. ailanpalai*, *E. caeruleocauda* tend to use more vertical habitat structure, exposing them to predation by all size classes of snakes, not just larger ones more prone to foraging terrestrially.

The two other lizards appearing in snake stomach contents were *A. carolinensis* and the Mangrove Monitor, *Varanus indicus*. Introduced to Guam in the mid-1950s, *A. carolinensis* was once common across the island, but since the irruption of Brown Treesnakes it has persisted in significant numbers only in urban habitats and appears to be particularly vulnerable to predation by snakes (Fritts and Rodda, 1998). Our results support this earlier finding: *A.*

carolinensis were only important dietary items at urban survey sites, particularly at URB2 where anoles accounted for 39% of diet items; this site was more isolated from forest habitats than the other two urban sites. Five cases of predation on *V. indicus* were observed in this study. The relationship of *V. indicus* to Brown Treesnakes appears to be one of infrequent and opportunistic reciprocal predation, in that snakes occasionally take small monitors and monitors occasionally consume snakes, with neither having any obvious suppressive effect on abundance or distribution of the other. All small lizard species on Guam have been negatively affected by Brown Treesnake predation, some to the point of extirpation (Rodda and Fritts, 1992). While *H. frenatus, L. lugubris, C. ailanpalai,* and *E. caeruleocauda* remain remarkably abundant on Guam, manipulative experiments have demonstrated that predation continues to have a top-down effect on small lizard densities (Campbell, III et al., 2012). This prevailing hyperabundance of small lizards may have enabled Brown Treesnakes to persist at high enough densities to enable hyperpredation (*sensu* Holt, 1977, and Smith and Quin, 1996) on Guam's native forest birds to the point of their extirpation (Savidge, 1987).

Frogs – Guam has no native amphibians. Aside from introduced Marine Toads, *Rhinella marina,* all abundant anurans on Guam were likely introduced since 2000 (Christy et al., 2007). Of these, tiny *E. planirostris,* and the much larger Günther's Frog, *Hylarana guentheri,* have become extremely abundant and widespread. We documented 24 *E. planirostris,* 2 *H. guentheri* and 1 Hong Kong Whipping Frog, *Polypedates megacephalus,* in our sample. Ours is the first substantive account of the role of frogs in Brown Treesnake diets on Guam. These frogs accounted for 5.7% of prey items in our sample, a proportion indistinguishable from that within the native range (Greene, 1989; Trembath and Fearn, 2008; Figure 3.3). Prevalence of frogs in stomach contents was higher in forest habitats, particularly scrub and ravine forests. This rate of frog predation on Guam is likely to have begun only within the last decade. Given the paucity of larger bird and mammal prey items and increasing abundance of larger frogs, particularly *H. guentheri,* it is conceivable that frogs may become

a larger portion of Brown Treesnake diets. Increased capitalization on this food source could have detrimental consequences for snake population management.

Overall, the majority of species preyed upon by Brown Treesnakes on Guam are non-native, reflecting an "invasional meltdown" (Simberloff and Von Holle, 1999) resulting from multiple invasions of non-native species and extirpations of native species. The general pattern of prey persistence seems to be that native species of lower reproductive capacity and lacking coevolutionary history with snake predators (i.e., the native forest birds) have been completely extirpated while introduced domestic and commensal birds and mammals persist with occasional predation, primarily in savanna and urban habitats which are sub-optimal for snakes. Species with high reproductive capacity, particularly native and non-native small lizards, persist at high abundance in forest habitats despite some suppressive effect of predation (Campbell, III et al., 2012). The paucity of larger endothermic prey resources in forests suggests that snakes in these habitats may have diminished resources for growth and reproduction compared to snakes in savanna and urban habitats.

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Chapter Four: Invasive Brown Treesnake Movements at Road Edges Indicate Road Crossing Avoidance[1]

Introduction

Roads have significant impacts on wildlife populations, including but not limited to direct mortality from vehicle-animal collision, reduction and fragmentation of habitat, acoustic disturbance, providing avenues of ingress for invasive species, and barriers to migration, resource selection and gene flow (Forman and Alexander, 1998; Spellerberg, 1998; Forman et al., 2003). These effects have been demonstrated in a diversity of taxonomic classes. With reference to herpetofauna, roads have been reported as barriers to dispersal or sources of direct traffic mortality for entire amphibian and reptile assemblages (Vijayakumar et al., 2001; Glista et al., 2008; Eigenbrod et al., 2009), as well as Timber Rattlesnakes (*Crotalus horridus*; Clark et al., 2010), Eastern Massasauga (*Sistrurus catenatus catenatus*; Shepard et al., 2008a,b), Black Ratsnakes (*Elaphe obsoleta obsoleta*; Row et al., 2007), Red-sided Gartersnakes (*Thamnophis sirtalis parietalis*; Shine et al., 2004), and vipers (*Vipera latastei* and *V. seoanei*; Brito and Álvares, 2004). Andrews and Gibbons (2005) found that nine snake species exhibited some aversion to crossing roads when soft-released at road edges, and no species demonstrated crossing trajectories that significantly differed from perpendicular to the road, indicating that snakes were attempting to minimize the distance necessary to leave the road.

Although roads typically represent impediments to biodiversity conservation, they may present opportunities for invasive species managers because roads are often expedient boundaries for demarcation of management units. For instance, habitat fragments delimited by roads are obvious management units for landscape-scale suppression of invaders. Roads may slow the spread of invasive species that avoid crossing them, while simultaneously providing

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access for field personnel deploying suppression technologies.

The accidental introduction of the Brown Treesnake (*Boiga irregularis*) to the island of Guam has had profound and cascading ecological and economic consequences (Savidge, 1987; Rodda et al., 1997; Wiles et al., 2003; Rodda and Savidge, 2007; Rogers et al., 2012). Reducing the probability of accidental export of Brown Treesnakes from Guam to other snake-free islands in the Pacific and restoring key habitat for the potential reintroduction of extirpated species are currently the primary management objectives. One approach to achieving these objectives is the targeted application of snake suppression technologies on a landscape level (Savarie et al., 2001; Shivik et al., 2002). As control scenarios expand to encompass significant portions of Guam, an understanding of the landscape-level movement of snakes will become increasingly relevant. Further, should Brown Treesnakes be accidentally introduced to neighboring snake-free islands (e.g., Saipan, Rota, Tinian), information about impediments to landscape-scale dispersal may be valuable in predicting and mitigating spread and coordinating eradication efforts.

Although Brown Treesnakes are largely arboreal, previous studies have documented that individuals occasionally cross roads (Santana-Bendix, 1994; Tobin et al., 1999; Savarie et al., 2001) and suffer mortality due to roadkill (McCoid and Hensley, 2000). Although roads are not impermeable barriers to Brown Treesnake movement, they may have some effect of slowing their dispersal on a landscape scale.

Guam is reticulated by a network of over 1700 km of road, with 46% of Guam's circa 520 km^2 within 100 m of a road (Siers, unpub. data). The movement of snakes from one category of land management to another is of most immediate management concern. Examples include movement from privately-owned lands to military facilities, into snake suppression zones from areas not under suppression measures, from forest sites to high-risk transportation hubs (airports and seaports), and other transitions among categories of land management. For these reasons, it is of significant management interest to assess whether Brown Treesnakes avoid crossing roads, and what factors modulate the rates at which they

do cross roads. It is worthwhile to distinguish between the terms "road avoidance" and what we are calling "road crossing avoidance." In addition to the effect of roads as barriers to movement, road avoidance may also describe the degradation or loss of habitat associated with proximity to a road, which is considered to be one of the primary effects of roads on animal populations (Fahrig and Rytwinski, 2009; Benitez-Lopez et al., 2010). However, regarding Brown Treesnakes on Guam, there is no evidence that roadside habitat is sub-optimal. Indeed, nighttime surveys of forest edge along roadsides is the most productive method for visual detection of this nocturnal species (Rodda and Fritts, 1992). For this reason, we choose to use the more specific terminology of "road crossing avoidance."

Because Brown Treesnakes have colonized all of Guam's habitats, we assume snake movement is a result of individuals seeking to maximize encounters with prey and/or potential mates, thermoregulate, find nesting sites, and avoid predation in a near-equilibrium environment. In the absence of diffusion gradients (e.g., snakes moving into unoccupied habitats), random movement is a logical null hypothesis by which to assess the effects of habitat and landscape features on animal movement. Random walk simulations are the most widely used animal movement models (Codling et al., 2008) and have been used as null hypotheses for animal movement in relation to roads in a variety of taxa, including elk (*Cervus elaphus*; Friar et al., 2008), Eurasian lynx (*Lynx lynx*; Kramer-Schadt et al., 2004), hedgehog (*Erinaceus europaeus*; Rondinini and Doncaster, 2002), Land Mullet (a large skink, *Egernia major*; Klingenböck et al., 2000), and multiple turtle species (Gibbs and Shriver, 2002; Shepard et al., 2008b). Among snakes, 40 radio-tracked Eastern Massasauga (*S. c. catenatus*) crossed roads less than expected by random walk simulations (though most P-values were > 0.05; Shepard et al., 2008b), while crossings by 68 Black Ratsnakes (*E. o. obsoleta*) did not significantly differ from random walk predictions (Row et al., 2007). Although Black Ratsnakes do frequently utilize arboreal habitat, to our knowledge there have been no other investigations as to the effect of roads on the movements of largely arboreal snake species, nor of any other tropical snakes.

As a first step in assessing the factors that influence the rates at which Brown Treesnakes cross roads, we use random walk simulations to test the null hypothesis that roads and road edges have no effect on the movements of this species.

Methods

Between 14 June and 1 August 2011, I conducted trials in which I monitored radio-tagged Brown Treesnakes for road crossing. A successful trial consisted of two consecutive daily location estimates of a transmitter known to be in a snake, achieved by triangulation of signals from VHF radio transmitters, whether or not there was perceptible movement of the snake. I considered a location on the opposite side of the road from the previous day's location as a crossing. Because Brown Treesnakes are nocturnal and hide in refugia during daytime hours, successive daytime location estimates appropriately reflect daily relocation activity.

Telemetry Methods — I conducted trials along two separate ∼300-m straight road segments on the Marbo Annex of Andersen Air Force Base on Guam. Site 1 was an east-west oriented segment of mowed-grass road following a power line, with secondary forest habitat on each side of the road. The mean distance between habitat edges, or gap width, was 10.03 m (SD = 0.83 m). The geographic center point of the road segment was at 1494837N and 268760E (UTM Zone 55N, datum WGS 84). Site 2 was a two-lane paved road with mowed shoulders oriented north to south and centered at 1495100N and 270488E. The road surface width was 6.3 m and the gap width between habitat edges was 11.67 m (SD = 0.51). The habitat was denser and taller secondary forest mixed with native forest. These two sites were approximately 1.75 km apart and separated by a largely homogeneous tract of secondary forest; I chose the sites to be paired in habitat, geographic region, climatic conditions, and snake population characteristics, so that differences in crossing rates could be attributed to differences in road characteristics (width, paved versus unpaved, traffic levels). I recorded traffic rates with a pneumatically-activated traffic counter (5600 Series Roadside

Unit, MetroCount, Fremantle, W.A., Australia). At both sites, I initially trapped snakes (Rodda et al., 1999) within 100 m of the road edge. While in the trap, most snakes voluntarily ingested a small dead mouse, weighing approximately 8 g, containing a 3.8-g Holohil PD-2 transmitter (Holohil Systems Ltd., Carp, Ontario, Canada). In two cases of multiple snakes captured in a single trap, I coerced snakes to ingest a transmitter by placing it in the mouth and massaging it down the esophagus. Because Brown Treesnake traps are known to yield size distributions biased toward larger snakes (Rodda et al., 2007), at Site 2 I sought to add smaller size classes by supplementing our trapping efforts with snakes hand-captured during visual searches; seventeen hand-captured snakes were coerced to ingest size-appropriate transmitters (Holohil, 0.51-g BD-2N, 1.8-g BD-2, or 3.8-g PD-2). I chose small transmitters and bait mice to minimize any potential satiation effects on snake movement (e.g., Lutterschmidt and Reinert, 1990). For the 31 snakes that ingested a transmitter and mouse, the mean ingested weight was 12.5% of snake body mass (SD = 3.1%); transmitters weighed 2.7% of snake body mass (SD = 0.7%) for the 19 snakes fed only transmitters. Actively foraging Brown Treesnakes are frequently caught with relative mass of gut contents exceeding the relative mass of our transmitter and bait.

I recorded snout-vent length (SVL), weight, and sex from all captured snakes prior to release at the capture location. I obtained daily locations for each snake by triangulation on transmitter signals using a null peak antenna array (Precision Direction Finding Antenna RA-4-NS, Telonics, Inc., Mesa, Arizona, USA) consisting of two 3-element yagi antennae mounted in parallel on a 1.8-m cross-mast. To maximize signal reception and avoid signal bounce from the ground, the array was mounted atop a portable 5.2-m aluminum mast. I recorded bearings from multiple telemetry stations with pre-recorded GPS coordinates. Maximum likelihood estimates of the signal location were obtained using the program LOAS™ 4.0 (Ecological Software Solutions LLC, Hegymagas, Hungary). If any bearings obtained with the mast-mounted antenna could not unambiguously determine the side of the road on which the snake was located, I tracked the transmitter signal with a handheld antenna to

determine the side of the road with absolute certainty.

For each daily trial, I recorded a movement distance as the Euclidean distance between the estimated location at the start of the trial and at the end ($\sim$24 hr later). This distance is an estimate of the relocation distance between successive daytime refugia, and does not include the full movement path of the snake. Clark (1998) and Tobin et al. (1999) demonstrated that the cumulative distance traveled by Brown Treesnakes was 2–4 times the nightly relocation distance. It is possible that a snake crossed a road during the true movement path and returned to the original side of the road on the same night, whereby I would have missed two road crossings; I assume this does not happen regularly and that true relocation of a snake's activity area would be adequately captured by our daily monitoring. Snakes were infrequently observed on roads or road shoulders during intensive island-wide visual surveys (Siers, pers. obs.), supporting my contention that such back-and-forth movement is likely to be rare, with little influence on the results of this study. All animal use was conducted in accordance with Colorado State University IACUC Protocol #11-2592A.

Random Walk Simulations— My random walk simulations used the actual starting location and relocation distances observed for each snake at each site. However, rather than recreating the observed movement path – with observed turning angles between each daily relocation – the turning angle was drawn randomly from a uniform distribution. Randomizing the turning angle simulates a utilization of the habitat space that is completely random with respect to any habitat features.

I conducted simulations using the statistical programming environment R, version 2.13.0 (R Development Core Team). As each road segment tested was straight, it was sufficiently represented by the equation of a line intersecting two geographical points along the road (in units of meters north and east as per the UTM Zone 55 N coordinates system). For each simulated movement path, the starting location for an individual snake, as actually observed, was plotted in the coordinate space. A successive location was simulated by assigning the first observed movement length a random turning angle and plotting the resulting location.

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From the newly simulated snake location, the procedure was repeated using the second observed movement length, and so on, until the complete observed movement history of the snake had been recreated based on random turning angles; see Figures 4.1 and 4.2 for graphical comparison of observed and randomized movement paths. I recorded the number of times each simulated movement crossed the road and duplicated this process for each snake at each site, summing all observed crossings by site. I repeated this procedure for a total of 5,000 iterations for each site. I represented the distribution of the number of road crossings predicted by the random walk simulation process with a histogram of the total number of crossings recorded for each iteration of the simulation. An empirically-observed number of crossings higher than the 97.5[th] percentile of this distribution would demonstrate greater-than-random road crossing at $\alpha = 0.05$; a number of observed crossings lower than the 2.5[th] percentile would demonstrate lower road crossing rates than expected by random movement with respect to roads.

Figure 4.1: Comparison of observed movement paths (left) and a single iteration of the simulation with randomized turning angles (right) for all snakes tracked at Site 1. Black dots indicate the initial location of the snake, and the gray line represents the focal road segment. Axis units are meters in the UTM Zone 55 N geographic coordinate system.
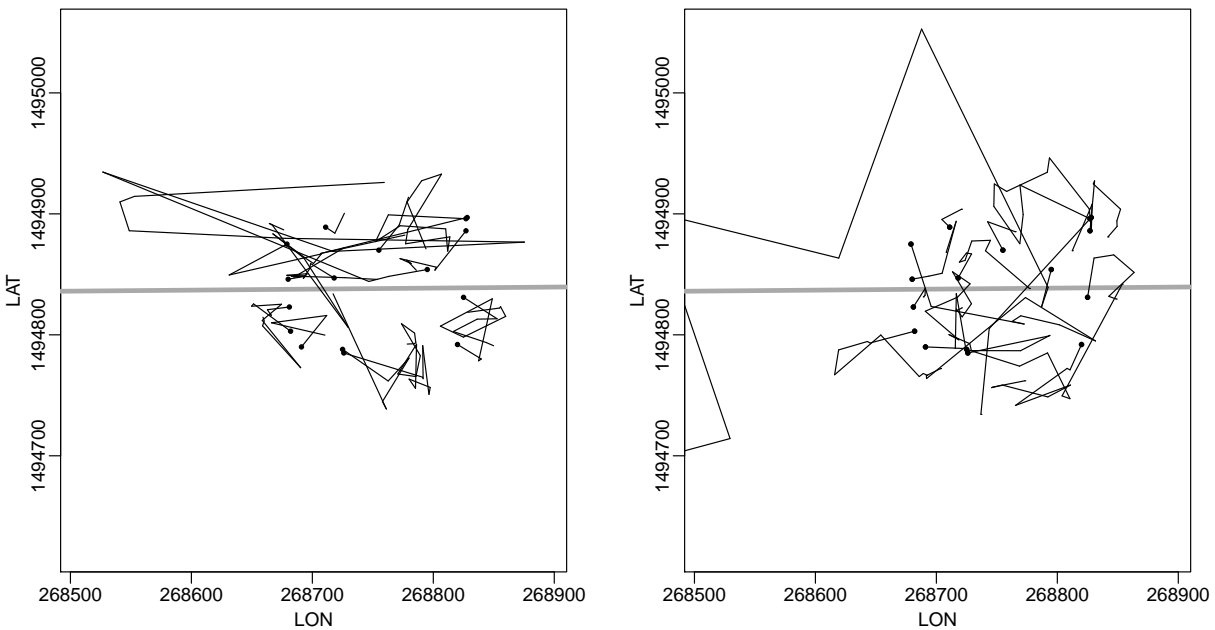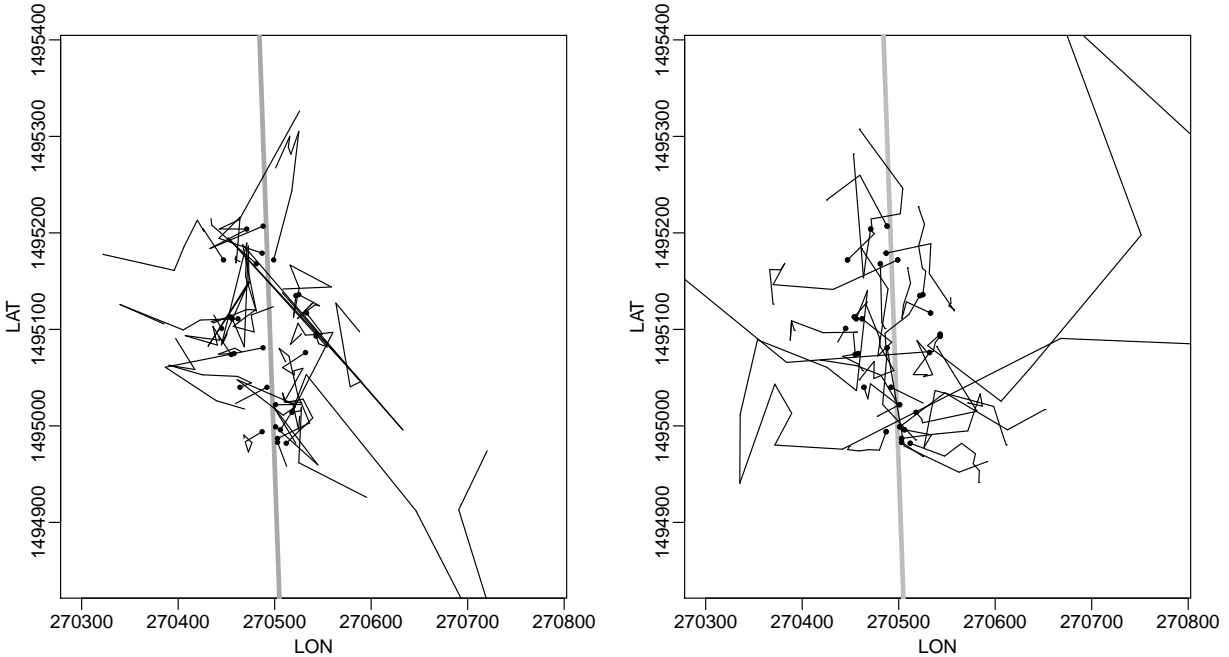
Figure 4.2: Comparison of observed movement paths (left) and a single iteration of the simulation with randomized turning angles (right) for all snakes tracked at Site 2. Black dots indicate the initial location of the snake, and the gray line represents the focal road segment. Axis units are meters in the UTM Zone 55 N geographic coordinate system.

Results

I tracked 20 trapped snakes at Site 1 and 30 snakes (19 trapped, 11 hand-captured) at Site 2. Because transmitters were ingested, transit times through the gut limited the number of trials obtained per snake. For the 50 snakes included in our trials, transmitter retention ranged from 1 to 17 days, with a mean of 7.2 days (SD = 3.5). Because not all individuals were successfully located each day, the actual number of trials recorded per individual ranged from 0 to 16, with a mean of 5.3 trials (SD = 3.3). To eliminate ambiguity associated with missing location estimates, we only included complete, uninterrupted tracking sequences, and eliminated all data subsequent to a missing observation. Our final dataset included 242 successful trials of 45 snakes (105 trials of 16 snakes at Site 1 and 137 trials of 29 snakes at Site 2). At Site 1, trapped snakes had a mean SVL of 954 mm (range 820 to 1130 mm) and consisted of 6 males and 10 females. At Site 2, trapped snakes and smaller hand-captured

snakes had a mean SVL of 914 mm (range 581 to 1,117), including 13 males, 11 females, and 5 snakes too small to be reliably sexed.

At Site 1, during the monitoring period of 14 June 2011 through 3 July 2011, rainfall averaged 6.85 mm per night (SD = 7.45) and the mean number of vehicles passing between 1900 and 0600 hrs (the hours of darkness, the presumed primary activity period for this nocturnal snake) was 0.1 (SD = 0.25) per night. At Site 2, precipitation averaged 14.44 mm per night (SD = 21.4) during the monitoring period (19 to 30 July 2011), and traffic averaged 12.6 vehicles per night (SD = 4.88).

From 242 successful field trials, I observed only 9 crossings: 2 crossings in 105 trials at Site 1 and 7 crossings in 137 trials at Site 2. The 2 observed crossings at Site 1 were by a single female (SVL = 916 mm), while the 7 crossings at Site 2 were by 2 males (SVL = 970 and 1035 mm) and 3 females (SVL = 793, 1068, and 1069 mm; the 793 mm and 1069 mm females each crossed twice). Although snake size, body condition, and sex were recorded, I did not seek correlations with relative road avoidance due to the small number of crossings.

Relocation distances ranged from 1.2 to 253.7 m (mean = 38.4, SD = 44.5). The relocation distances for the 9 road crossing events ranged from 24.6 to 253.7 m (mean = 117.7, SD = 70.4). These movements were estimated based on distances between GPS locations (positional accuracy ±3 m) or triangulated locations of radio transmitters (median variance = 4.28 m).

I depicted the distributions of the numbers of crossings predicted by random walk simulations for each site in Figure 4.3. The mean number of predicted crossings for Site 1 was 12 (95% CI: 5–20); for Site 2, the mean was 20 (95% CI: 12–30). At Site 1, only 12 of 5,000 iterations predicted 2 or fewer crossings, equivalent to P = 0.0024. At Site 2, only 4 iterations predicted 7 or fewer crossings for P = 0.0008.

To test for potential effects of satiety, I conducted a regression of average movement distances for each snake against the proportion of bait and/or transmitter mass to snake mass, revealing no significant negative effect (P = 0.23), and the relationship explained less
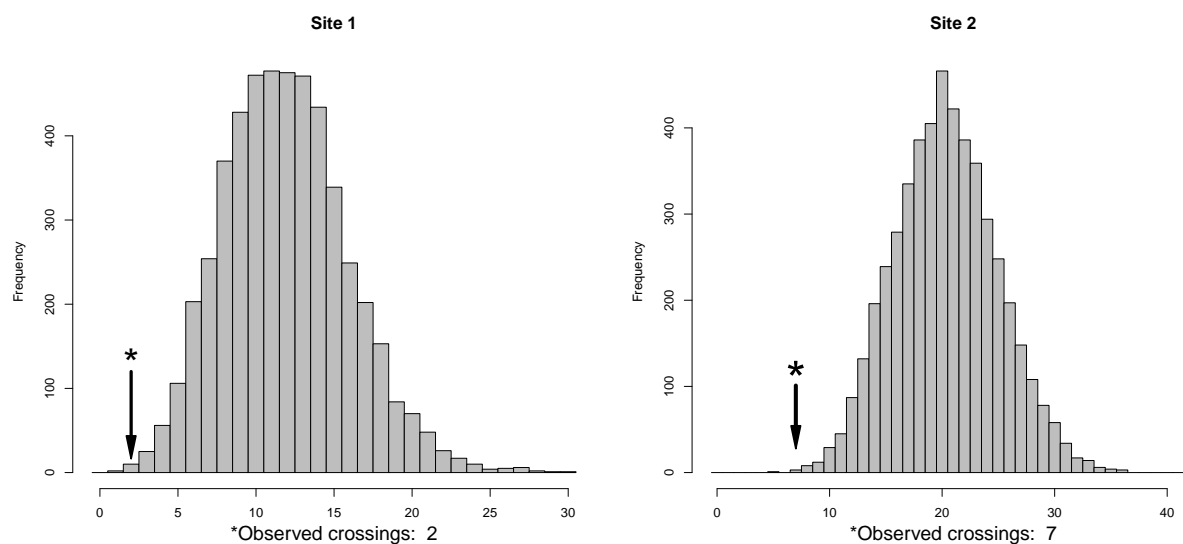
Figure 4.3: Histograms of the numbers of road crossings predicted by 5,000 iterations of a random walk simulation. Arrows and asterisks denote the value of the actual number of crossings empirically observed during field trials.

than 1% of the variation in movement distances.

Discussion

Brown Treesnakes do not appear to utilize Guam's landscape in a random fashion with respect to roads, resulting in a lower than expected rate of road crossing when compared to a random walk null model. At our two sites, the mean predicted rates of crossing under the assumption of random movement were six times higher than the observed crossing rate at Site 1 and nearly three times higher at Site 2.

The greater road crossing rates at Site 2, a paved road with higher traffic levels, than at Site 1 are likely an artifact of our sampling methods. Although all snakes at Site 1 were captured in traps as far as 100 m into the forest interior, Site 2 traps were closer to the road (within 50 m) and hand-captured snakes were found directly on the forest edge, resulting in a higher probability of a randomized angle leading to a road crossing at Site 2. Sampling closer to the road edge increases our power to detect non-randomness in movements, as

simulated movements following randomized turning angles are more likely to cross a road if it is in close proximity; sampling Site 1 closer to the road would have resulted in even greater statistical support for our results in that the number of simulated road crossings would shift toward higher expected values, leaving our observed result even further in the lower tail of the frequency distribution (see Figure 4.3).

Visual examination of the observed movement paths of snakes (left panes of Figures 4.1 and 4.2) suggests that much of the movement was parallel to road edges. However, movement into the dense forest interior (perpendicular to the road) increased the likelihood that we would fail to detect a signal from the transmitter. Once a signal was lost, subsequent movements for that snake were not considered in the random walk models. Elimination of movement data subsequent to missing observations did not eliminate any observed crossings. Inclusion of such data would increase the number of simulated movements, decreasing the per-observation road crossing rate, and would increase the statistical significance of the results.

Telemetry data inherently have associated error, and ignoring such error can have serious inferential consequences in many telemetry studies (Saltz, 1994; Montgomery et al., 2011). Because relocation distances were the distances between two successive location estimates, there would be some associated error; however, this error would likely only add noise to the observed movement lengths and have little influence on the overall amount of movement. Because turning angles are randomized in the simulation procedure, and the telemetry errors are not likely to bias movement distances in a systematic fashion, I believe that telemetry uncertainty is unlikely to introduce bias in the random walks. Observed road crossing rates would not be biased by telemetry error, as I unambiguously determined which side of the road the snake was on.

Potential effects of satiety (diminished movement due to the stomach contents comprised of a transmitter and in most cases a small mouse) did not appear to bias our results. The observed movement distances were similar to those found in previous telemetry work that

was based on surgically-implanted transmitters (Santana-Bendix, 1994; Tobin et al., 1999). The notion that recent feeding success decreases movement rates may be more plausible in sit-and-wait predators taking larger meals, rather than actively-foraging species subsisting on smaller prey (Secor, 1995).

There are at least three possible hypotheses to explain low rates of road crossing of Brown Treesnakes on Guam. My initial hypothesis was that the snakes may be deterred by physical and behavioral impediments to movement across roads. This could be related to Brown Treesnake preference for arboreal locomotion, particularly at small sizes (Rodda and Reed, 2007), or the risk of predation perceived by the snake when leaving protective cover and ample refugia offered by forested habitat. A snake's reluctance to cross a road may also be in response to passing vehicular traffic. Andrews and Gibbons (2005) reported differences in immobilization response ("freezing") in reaction to staged encounters with moving vehicles among three different snake species; how Brown Treesnakes respond to passing vehicles and whether vehicular encounters deter crossing attempts are unknown. An alternative hypothesis, which is not necessarily mutually exclusive, is that prey are more abundant in the forest vegetation at road edges and snakes are reluctant to leave such successful foraging locations. Dense leafy vegetation at the edge of the forest understory may provide more structure, cover, and insect prey for small lizards (primarily geckos and skinks), which currently dominate the diet of Brown Treesnake populations on Guam (Savidge, 1988; Chapter Three). Our results demonstrate an apparent avoidance of road crossing, but do not distinguish between these explanatory hypotheses.

One might argue that observed movement paths differ from randomized paths in that snakes are adhering to a foraging area or "home range," and that site fidelity, possibly due to prey availability, explains the low rates of observed road crossings. Tobin et al. (1999) found that Brown Treesnakes on Guam concentrated their activities in core areas, and mean relocation distances of 60 snakes after 60–70 days were only approximately 70–90 m. Conversely, Santana-Bendix (1994) tracked 11 Brown Treesnakes for 35–103 days, and

although there were short periods with smaller activity areas, longer movements to new areas continued to increase the cumulative activity area with no asymptote. He concluded that the species lacks defined activity areas and moves randomly and continuously. Regardless of the support for defined activity areas for Brown Treesnakes, it is clear that such areas are not randomly distributed across the landscape with respect to roads. Whether apparent road crossing avoidance manifests through exclusion of roads within home ranges, reluctance to leave areas of higher prey density, or avoiding habitat gaps in the course of otherwise random movement, the magnitude of the effect demonstrated here remains the same.

The forest habitat utilized in this study is fairly representative of a large portion of Guam's landscape, including key areas for snake suppression and potential recovery of native species. Although my road sites were assumed to be at equilibrium with respect to prey and competitor densities, in management zones where snakes are suppressed and prey communities begin to recover there is potential that snakes, motivated by greater prey density or lower competitor density, may cross roads at a rate greater than observed here. On islands where novel invasions by Brown Treesnakes may occur, roads may slow the invasion front only until shifts in prey and competitor densities motivate snakes to cross roads.

I demonstrated that road edges are not neutral landscape features with respect to Brown Treesnake movement, and provided managers with a quantitative baseline assessment of the effects that roads and road edge habitats have on influencing landscape-level movement of this costly invasive predator on Guam. The methods I employed may be useful for others investigating road crossing rates by wildlife.

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Chapter Five: Intrinsic and Extrinsic Factors Influencing Road Crossings by Invasive Brown Treesnakes on Guam

Introduction

Roads are pervasive landscape features with the potential to have severe impacts on a diversity of wildlife taxa. Road effects include mortality from road construction, mortality from vehicle collision, alteration of the physical and chemical environment, spread of exotic species, increased human use, and modification of animal behavior including a barrier effect to landscape-level movements (Trombulak and Frissell, 2000). The effects of roads on wildlife have intrinsic components, pertaining to the abilities and behaviors inherent to the individual or species, and extrinsic components, modulating animal behavior in response to characteristics of the road and the ecological context (Forman et al., 2003; Andrews and Gibbons, 2005). Effects of these components can vary among and within taxa (Benitez-Lopez et al., 2010), with some individuals or species more vulnerable to the detrimental effects of roads (Hels and Buchwald, 2001; Fahrig and Rytwinski, 2009). Similarly, variability in road characteristics and ecological context can lead to spatial or temporal "hotspots" of road mortality or "cold spots" where the transportation infrastructure is impermeable to wildlife movement (Lewis et al., 2011; Beasley et al., 2013; Beyer et al., 2013; Crawford et al., 2014). Within the flourishing subdiscipline of road ecology, however, rarely are both intrinsic and extrinsic factors explicitly and quantitatively assessed within the same system.

The barrier effect of roads on animal movements can range from on-road mortality (Hels and Buchwald, 2001) to lack of access to required resources (Law and Dickman, 1998), slowing of migratory movements (Bouchard et al., 2009), loss of gene flow (Balkenhol and Waits, 2009), and other effects of fragmentation (Saunders et al., 1991). Snakes can be particularly vulnerable to these effects (Bernardino and Dalrymple, 1992; Row et al., 2007; Shepard et al., 2008a), which may vary by species and be influenced by interactions among demographic, ecological, and temporal factors (Jochimsen et al., 2014). Conversely, while

roads may be detrimental for wildlife populations, any effects of roads on slowing movements of invasive species may be seen as advantageous from a management perspective.

The Brown Treesnake (*Boiga irregularis*) is native to northern and eastern Australia and many islands of Oceania. This rear-fanged, mildly-venomous colubrid snake is nocturnal, primarily arboreal, and a versatile generalist predator on small vertebrates. After its accidental introduction to the U.S. territory of Guam, circa 1945, it spread rapidly across the island, and by the mid-1980s all terrestrial habitats had been successfully invaded (Rodda et al., 1999b; Rodda and Savidge, 2007). This irruption was followed by a rapid decline in all of Guam's native terrestrial vertebrate fauna (Rodda et al., 1997). Native birds were particularly devastated, with the extirpation or extinction of nearly all forest avifauna (Savidge, 1987; Wiles et al., 2003). These losses have dramatically restructured Guam's food web (Fritts and Rodda, 1998) and have had cascading ecological effects (Mortensen et al., 2008; Rogers, 2011; Rogers et al., 2012). All prospects for the containment and suppression of this mobile species will be affected by its movement behavior, and roads are the most pervasive landscape feature that may pose partial barriers to movement. My previous work demonstrated Brown Treesnakes on Guam do not cross roads at the same rates as would be expected if they were neutral landscape features; in other words, they exhibit an apparent road crossing avoidance (Siers et al., 2014, Chapter Four). However, I did not assess the effects of intrinsic and extrinsic characteristics on road crossing avoidance or quantify road crossing rates. In this study, I describe the relative influence of a suite of intraspecific, contextual, environmental and road characteristics on per capita road crossing probabilities of this costly invasive predator.

Intrinsic factors. Variability in road crossing rates by Brown Treesnakes is likely to be influenced by characteristics of individual snakes, including:

Snout-vent length — Among snakes, large-bodied species are more likely to cross roads than small-bodied species (Andrews and Gibbons, 2005). Within some snake species, larger

individuals have larger home ranges and make longer and more frequent movements (Pizzatto et al., 2009; Hyslop et al., 2014). Increased movement activity increases the potential for contact with roads and road mortality associated with vehicle-snake collisions, and more vagile species and life stages may be particularly vulnerable (Jochimsen et al., 2014). In many species, larger snakes suffer higher road mortality (Brito and Álvares, 2004; Andrews and Gibbons, 2005). However, previous telemetry work on Brown Treesnakes did not show significant differences in movement rates (Santana-Bendix, 1994; Tobin et al., 1999) or road crossings (Tobin et al., 1999) by size class. Because larger Brown Treesnakes are more prone to terrestrial locomotion and foraging (Rodda and Reed, 2007), if there is a correlation with snout-vent length (SVL), I predict that larger snakes will cross roads more frequently.

Sex – Males of many species of snake have significantly larger home ranges and move further and more frequently than females (Pearson et al., 2005; Richardson et al., 2006; Glaudas et al., 2008; Hyslop et al., 2014), and natal dispersal is often demonstrated to be male-biased (Keogh et al., 2007; Dubey et al., 2008). Greater use of roads by males may lead to them being found dead on roads at higher rates than females (Andrews and Gibbons, 2008; Shepard et al., 2008a; DeGregorio et al., 2010). Capture-mark-recapture studies of a geographically closed population of Brown Treesnakes on Guam indicate higher detection probabilities for males (Christy et al., 2010), which could be a result of greater movement activity. However, previous work on Brown Treesnakes showed minimal or non-significant sex differences in measures of movement (Santana-Bendix, 1994; Tobin et al., 1999), road crossing (Tobin et al., 1999), foraging ecology (Savidge, 1988), and effectiveness of control tools (Tyrrell et al., 2009; Lardner et al., 2013). Therefore, I do not expect to observe sex differences in road crossing rates, but if they exist, I would predict them to be male-biased based on the general trend of higher movement rates and road use by males of other snake species.

Reproductive class – Reproductive activities strongly influence snake movements (Gregory et al., 1987). Differences in movement among male and female snakes are often the

result of the reproductive status of the individual. Reproductive males may have larger home ranges (Shine, 1987; Marshall et al., 2006) and make longer, more frequent, and more bold movements while seeking mates, particularly during breeding seasons (Secor, 1995; Rouse et al., 2011). This may lead to more frequent interactions with roads and subsequent mortality (Shepard et al., 2008a). Reproductive female snakes are often known to move less or cease movement completely while gravid (Seigel et al., 1987; Charland and Gregory, 1995; DeGregorio et al., 2011) or tending a clutch (Pearson et al., 2005), but may make particularly long movements before (Brown et al., 2005) or after (DeGregorio et al., 2011) oviposition. Brown Treesnakes are typically grouped into reproductive categories based on size class as determined by Savidge et al. (2007); however, aside from obviously gravid females, actual reproductive status (oogenesis and parturition history, spermatogenesis) is impossible to fully determine without necropsy and microscopy. Given the general trends in other snakes and our inability to accurately assess true reproductive status, I suggest that if there is an effect, juvenile snakes ($<$ 910 mm for females and 940 for males) and snakes of intermediate sex (910 to 1025 and 940 to 1030 mm) will cross roads more than mature females ($>$ 1025 mm) and less than mature males ($>$ 1030 mm).

Body condition – Body length to mass ratios are considered to be good indicators of nutritional status and metabolic reserves in snakes (Bonnet and Naulleau, 1996). Animals in poor body condition may forage more and be less vigilant (Bachman, 1993), and therefore more prone to risking cross-road movements. However, longer movements may require more metabolic stores, so snakes in low body condition may opt for a sit-and-wait foraging mode rather than actively foraging, resulting in lower crossing rates for snakes in poor condition. Upon experimental suppression of rodent prey on Guam, Brown Treesnakes in poor body condition exhibited greater site fidelity (Gragg et al., 2007) and individuals in better body condition moved more irrespective of plot treatment (Christy et al., unpub. data). Based on these results, I would predict greater road crossing rates by snakes in better body condition.

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Extrinsic factors.   In addition to characteristics of the snake, factors external to the snake can play a role in influencing a snake's decision to cross a road:

Road characteristics – Physical or behavioral road barrier effects and on-road mortality are likely to be influenced by factors such as gap width between habitat edges (Tremblay and St Clair, 2009; van der Ree et al., 2010) and road surface type (e.g., paved or unpaved; Fortney et al., 2012; Robson and Blouin-Demers, 2013; Hyslop et al., 2014). Reconstructing long-term Brown Treesnake movement paths reported by Santana-Bendix (1994), I noted that crossings of unpaved roads were nearly four times as frequent as crossings of paved roads (Siers, unpub.). Road traffic levels also have a strong deterrent effect on cross-road movements of snakes and other wildlife (Alexander et al., 2005; Andrews and Gibbons, 2005; McCown et al., 2009) and increase the risk of on-road mortality (Sutherland et al., 2010). I predicted that all measures of road magnitude (e.g., gap width, surface type, traffic volume) would have negative effects on the crossing rates of Brown Treesnakes.

Contextual factors – Some circumstances affecting road crossing decisions vary on a daily basis. In addition to average traffic volumes characteristic of a given road, traffic levels may vary on a daily and nightly basis. Road crossing avoidance (Reijnen et al., 1995; Andrews and Gibbons, 2005; Zurcher et al., 2010) and on-road mortality (Mazerolle, 2004) may therefore vary on a daily basis, and I hypothesized that road crossings by Brown Treesnakes would be negatively associated with higher than average nightly traffic volumes. I hypothesized a "distance effect," assuming that a snake close to a road at the beginning of its nightly movements would be more likely to cross that road than a snake beginning further away. As part of my snake collection methods (see below), snake traps baited with live mice were present along road edges for a variable number of nights until an adequate sample was achieved. Throughout multiple Brown Treesnake research projects we have observed snakes persistently yet unsuccessfully attempting to enter traps. I hypothesized that such an attractant effect of trap lures would result in reduced road crossing rates by snakes on nights when traps were present.

Environmental factors – Many snake species exhibit dramatic seasonal variation in activity areas and movement rates (Shine and Lambeck, 1985; Blouin-Demers and Weatherhead, 2001; Brito, 2003; McDonald, 2012). Increased seasonal movement may lead to increased road crossing and road mortality rates (Bernardino and Dalrymple, 1992; DeGregorio et al., 2010; Rahman et al., 2013). Guam's tropical climate is characterized by a warm, humid wet season and a cooler dry season, and metrics of Brown Treesnake activity (power outages caused by snakes in electrical infrastructure, envenomation reports and sighting rates) have historically been higher in the wet season (Rodda et al., 1999b). Because McCoid and Hensley (2000) reported higher rates of Brown Treesnake road mortality in Guam during the wet seasons of 1989 and 1990, I hypothesized a similar increase in road crossing rates during the wet season. In addition to seasonal variability, environmental factors may vary on a daily basis. Based on patterns suggested by literature on activity and movement of Brown Treesnakes and other snakes, I hypothesized a positive effect of temperature (Sperry et al., 2013), humidity (Daltry et al., 1998), and precipitation (McDonald, 2012) and a negative influence of wind (Christy et al., 2010) on road crossing rates. For nocturnal animals relying on darkness for predator avoidance and cryptic foraging, moonlight can have a negative effect on activity levels (Yamagishi, 1974; Lima and Dill, 1990; Griffin et al., 2005). Because small Brown Treesnakes have been documented to move less on nights directly following a full moon (Lardner et al., 2014), captive snakes used open areas in mesocosms less under high simulated moonlight (Campbell et al., 2008), and high moonlight decreased Brown Treesnake detection rates during visual surveys (Christy et al., 2010), I predicted a reduced probability of road crossing on nights with brighter moonlight.

By short-term radio-tracking of snakes in close proximity to roads, I sought to measure actual per capita road crossing rates and to assess the influence of nightly conditions and snake and road characteristics on crossing probabilities. Potentially influential factors and *a priori* hypotheses for their effects are summarized in Table 1.

Methods

Site selection – I selected 50 road, path, or utility corridor segments passing through forest habitats across the geographic expanse of Guam. Each of the selected segments was characterized by at least 200 m of relatively straight road or corridor with at least 100 m of forest depth on both sides. Occasional minor tree pruning at very narrow sites ensured that snakes could not cross gaps arboreally. I selected sites to be geographically dispersed (Figure 5.1) and to be representative of the full range of road or path types and traffic conditions occurring on the island (Figure 5.2). As an exception, we did not conduct trials along Guam's busiest roads out of safety considerations and due to the extremely low probability of observing crossings at such sites. Safety also necessitated that high-traffic roads have substantial shoulders from which to conduct operations.

Snake capture and processing – At each site to be surveyed, snakes were captured by funnel traps baited with live mice and by hand during nighttime visual surveys. We placed traps along both road edges and at depths of up to approximately 15 m from forest edge. Trapping continued for one to four nights until an adequate sample size of snakes was obtained. We conducted hand captures only on the first night at each site to avoid observers interfering with movements of snakes previously implanted with transmitters. Upon capture, we collected morphometric data including SVL, weight and sex. A lubricated VHF transmitter (Holohil Systems Ltd., Canada) was then gently massaged down the esophagus and into the stomach (Figure 3). Most snakes under 650 mm SVL received a 0.51-g model BD-2N, those from approximately 650 to 950 mm received a 1.8-g BD-2, and those over 950 mm received a 3.8-g PD-2. We then released snakes at the location of capture.

Telemetry methods – After release, I relocated snakes on a daily basis via triangulation with a null peak antenna array (Precision Direction Finding Antenna RA-4-NS, Telonics, Inc., Mesa, AZ) consisting of two three-element Yagi antennae mounted in parallel on a 1.8-m cross-mast atop a portable 5.2-m aluminum center mast. I recorded bearings from multiple triangulation stations with prerecorded GPS locations, with additional bearings from *ad hoc*
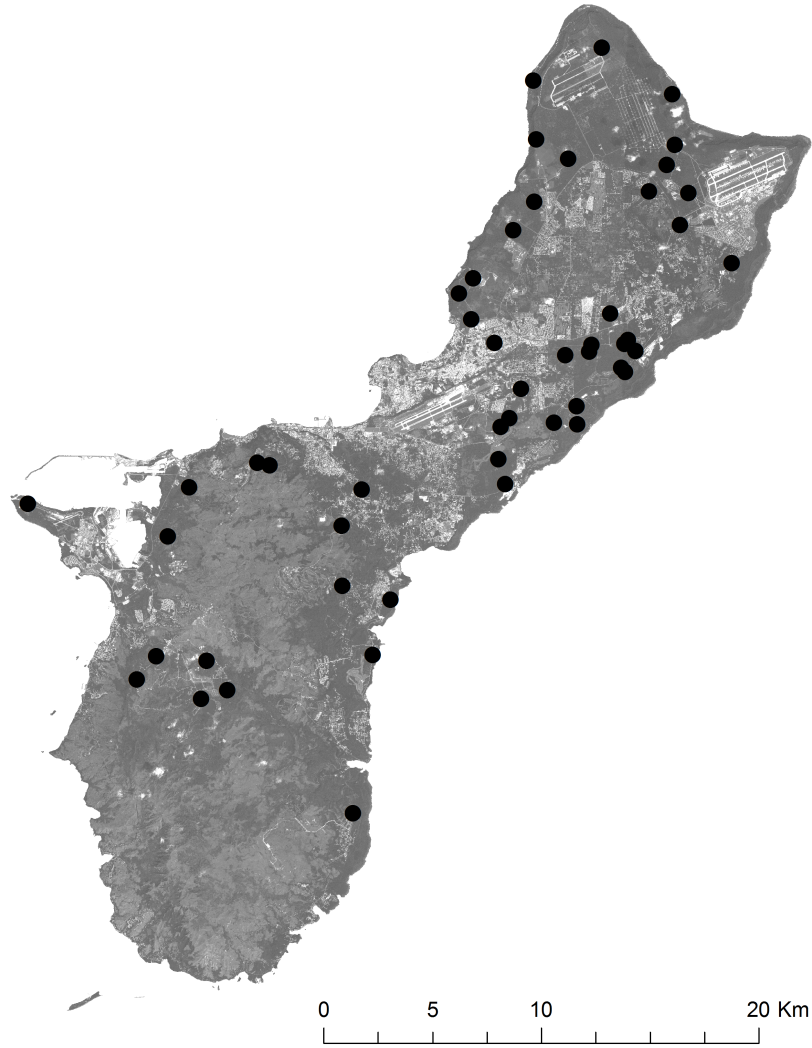
Figure 5.1: Map of the U.S. territory of Guam depicting geographic distribution of the locations of 50 road, path or utility corridor segments monitored for crossings by radiotelemetered Brown Treesnakes.

GPS locations as required. Maximum-likelihood estimates of the signal location and error ellipses were obtained using the program LOAS™ 4.0 (Ecological Software Solutions LLC, Hungary). If any bearings obtained with the null peak antenna could not unambiguously determine which side of the road the signal was coming from, I used a handheld antenna to determine the correct side. I recorded locations during the daytime when the nocturnal snakes were in refugia. I considered the distance between two successive daily locations the nightly relocation distance. I tracked snakes for up to 9 days, during which time I recovered

Figure 5.2: Photographs depicting the variability in characteristics of roads, paths and utility corridors on Guam monitored for crossings by Brown Treesnakes.

Figure 5.3: Gastric implantation by massaging a lubricated VHF radio transmitter down the esophagus and into the stomach of the snake, immediately followed by release at the location of capture.

the majority of transmitters from the ground or vegetation after being evacuated by the snakes. Trials during which I observed no movement and the transmitter was recovered after being located in the same location as prior locations were dropped from the analysis under the assumption that the transmitter had been shed (i.e., only trials where the transmitter was known to be in a snake were included in the analyses). All animal use was conducted in accordance with Colorado State University IACUC Protocol #12-3628A.

Site-level effects – I considered several descriptors of road magnitude as predictors of road crossing rates. I calculated gap width (*gap*) as the average of approximately 16 measurements of the width between forest edges on opposite sides of the road along the entire segment. If forest edges were not discrete, I began measurements where vegetation exceeded ankle height. I also measured the minimum gap (*min*), the point along the road segment where the gap between forest edges was narrowest. Surface width (*srf*) was the width of the road surface at paved sites, with unpaved roads assigned a value of zero. I classified roads with a binary categorical variable (*pav*) to indicate whether the road was paved or unpaved. I obtained average traffic levels at each site (*atr*) by taking the mean of all nightly traffic counts from sunset to sunrise during the survey period as recorded with pneumatically-activated traffic counters (5600 Series Roadside Unit, Metrocount, Fremantle, Australia). To improve normality and equality of variance, I log-transformed all traffic counts. Because each road segment was tested in only one season (wet or dry), I considered season (*sea*) as a potential factor at the site level despite it not actually being a characteristic of the road. Additionally, to account for latent influence of unmeasured characteristics of individual sites, I included a random effect of site ([*site*]) in all models. Many sites had zero observed crossings by snakes, which led to a high variance among sites on the logit scale, resulting in an increased influence of the random effect and diminished influence of fixed effects. I elected to retain the site-level random effect and present conservative estimates of the significance of the fixed effects.

Snake-level effects – I measured snake body size, or snout-vent length (*svl*), by gen-

tly stretching snakes along a flexible tape. Preliminary exploratory modeling indicated no quadratic effect of SVL, which would suggest higher or lower crossing rates by mid-sized snakes, so a quadratic term (svl^2) was not considered in the model selection process. Sex (sex) was determined by probing (Reed and Tucker, 2012); a small number of snakes too small to be reliably sexed as male or female were classified into a third "unknown" category. I assigned each snake to a reproductive class (cls) of either juvenile, intermediate, adult female or adult male based upon SVL classifications reported by Savidge et al. (2007), as previously described in the introduction. Condition index (ci) represents the standardized residuals from a fourth-order polynomial fit of a log (mass) to log (SVL) regression of all individuals within the dataset. I considered including a random effect of snake ID to account for multiple repeated measures of individuals; however, due to a small number of observations per snake and a large number of snakes with "all-zero" observations (no crossings), I determined that individual variability beyond that described by the preceding fixed effects could not be known given the data.

Trial-level contextual effects – I obtained the "distance effect" covariate (dst), or the snake's distance from the road at the beginning of the trial, from coordinates recorded by GPS at release or estimated by triangulation. To assess whether the presence of snake traps reduce a snake's probability of crossing a road, I recorded a binary covariate (trp) to indicate whether or not traps were present at the survey location on the trial night. To test the effect of nightly traffic rate variation beyond the average recorded at the site level, I also considered the nightly log-transformed traffic count (ntr) as a trial-level covariate.

Trial-level environmental effects – I calculated humidity (hum), temperature (tmp), precipitation (pr), wind speed (wnd), and wind gust speed (gst) as the means of all hourly values between sunset and sunrise on the night of the trial as reported by the National Weather Service weather station at the Guam International Airport (http://www.nws.noaa.gov). I considered 24-hr precipitation values from temporary plastic rain gauges at the survey sites as an alternative measure of precipitation (prg). I obtained information on moon elevation

and apparent magnitude from the NASA Jet Propulsion Laboratory HORIZONS system (http://ssd.jpl.nasa.gov). I calculated moonlight values (mn) as sine (elevation/max. elevation $* 0.5 * \pi$) $*$ apparent magnitude $* -1$, with the sine function attenuating moonlight as it moved lower on the horizon and multiplication by -1 converting negative apparent magnitude values to positive values representing brighter moonlight. I also considered a cloud-adjusted moonlight score (cmn), "penalizing" moonlight values as $mn - (0.66 * mn * \text{cloud cover})$, where cloud cover was the mean hourly nighttime proportion of cloud cover from the National Weather Service data.

Statistical analysis – I considered each nightly relocation as a Bernoulli trial, with a response variable of 1 or 0 indicating whether the snake did or did not cross the road. I employed mixed-effects logistic regression using the 'glmer' function from the R package 'lme4' to assess the effects of covariates. I based model selection on Akaike's Information Criteria corrected for small sample size (AICc). I included the site-level random effect ($[site]$) in all models, with model selection conducted on possible combinations of level-specific fixed effects.

I approached model selection in a stepwise fashion, first considering all candidate site-level effects. Each term was first assessed for its individual fit in addition to the base model (random site effect only for the site-level selection process) before all possible combinations of candidate terms were considered in an AIC model selection and model-averaging process. I added the most influential effects to the base model and carried them over for selection of additional fixed effects at the next level. I repeated this process through the snake-level, trial-level contextual, and trial-level environmental subsets, finally arriving at a top model describing the most influential factors affecting road crossing probabilities. P-values for each term included in this top model are also reported in Table 5.1. We also estimated coefficients and standard errors from a simplified model to allow the reader (e.g., invasive species managers) to make crossing probability estimates based on their own data (Table 2).

I visualized effect sizes by plotting predicted logistic response curves and 95% confidence

intervals ($\pm$1.96*SE) for the most significant results. Standard procedures do not exist for estimating standard errors for model predictions with random effects, so these effect sizes and standard errors are from models containing the fixed effects only; I acknowledge that unmeasured site-level effects may introduce more uncertainty about the estimates. Because the absence of traps represents the natural condition, all representations of predicted effect sizes hold the predictions constant at the trapless state.

Model simplification and simulation of absolute road crossings – I also derived a simplified model based on easily measured significant predictor covariates that a managing agency might use to predict the probabilities of road crossings based on road and snake population characteristics. In addition to reporting the model formulation, coefficients and standard errors, and P-values, I used this model to simulate differences in absolute road crossing rates based on actual size distributions and random movement within 100m of a hypothetical road edge. I used actual size distributions from samples of 100 snakes collected at each of two sites surveyed during another project (Siers et al., unpub.), a limestone forest site on the Naval Magazine, Naval Base Guam, and an urban residential area in the village of Dededo. These sites varied in mean SVL, with the Naval Magazine sample averaging 740 mm and the Dededo site averaging 914 mm. Each sample of 100 snakes as assumed to be a hypothetical population of 20 snakes per hectare, randomly distributes as far as 100 m from the road edge along a 500-m segment of road. I predicted the per-capita per-night crossing probability for each snake – using the simplified fixed-effects model – based on a randomized distance from the road for each individual snake (0 to100 m), a gap width of 10 m, a humidity of 85%, and with no traps present, and using actual snake lengths from two samples. I made a random draw from a binomial distribution with the predicted nightly crossing probability to simulate whether the snake did or did not cross the road. I ran the nightly estimation procedure – re-randomizing the distance from the road – 30 times (one month) for each iteration, and summed up the numbers of predicted nightly crossings. I ran this monthly simulation for 1000 iterations, producing a distribution of simulated monthly road crossings for each site to

demonstrate differences in numbers of road crossings based on variation in size distributions among sites.

Results

I conducted trials at two pilot sites from 14 June 2011 to 1 August 2011, with trials at the remaining 48 sites occurring between 11 June 2012 and 29 May 2013. I implanted transmitters in a total of 728 snakes, of which 691 yielded successful trials. I tracked those snakes for 1 to 9 trials each (where trials equal consecutive days an individual was successfully located), with a mean of 4 trials per snake (SD = 2.2 trials), for a total of 2,785 trials. Throughout the trials, I recorded a total of 95 road crossings.

Snakes ranged from 381 to 1,525 mm SVL. Very small and very large snakes were under-represented, as both were difficult to obtain and track; small transmitters emitted weak signals and were often lost, and very large snakes were rare and often left the sites shortly after implantation. Approximately 80% of my trial observations were of snakes between 760 and 1,096 mm, with a mean of 943 mm (SD = 149 mm) SVL. The mean transmitter-to-snake mass ratio was 3.78% (SD = 1.48%).

Distance errors for GPS locations (release and transmitter recovery locations) were from ± 2 to ± 6m, depending on canopy interference. I estimated distance errors for triangulated locations as the distance between the estimated transmitter location and the edge of the estimated error ellipse (as plotted by LOAS$^{\text{TM}}$ 4.0) perpendicular to the forest edge. Distance errors were correlated to estimated distance from the edge ($R^2 = 0.36$, P $<< 0.001$), with an estimated distance of 5 m having an estimated error of ± 1.0 m, out to an estimated error of ± 31.2 m for a location estimated at 100 m from the road edge. These errors only add noise to the distance covariate (dst) and would not affect my estimates of road crossing rates as all locations were unambiguously assigned to the correct side of the road.

Crossings per site ranged from zero to eight, with no observed crossings at 20 (40%) of the sites. The variance associated with the random effect in the base model ([$site$]) was 0.769

(SD = 0.577); inclusion of the selected model terms reduced this variance to 0.333 (SD = 0.577).

Among the site-level effects (Table 5.1A), all measures of road magnitude were significantly negatively correlated with road crossing rates as predicted. When each factor was taken as a sole covariate in a fixed-effects model, all P-values were $<< 0.001$. When the site-level random effect was included, all P-values were ≤ 0.014 ("P_{sole}" column in Table 5.1) and all ΔAICc values were $\leq$ -3.9. Because most of these measures were autocorrelated, we selected only the most significant predictor (average nightly traffic volume, atr) and the most readily measurable metric of the physical magnitude of the road (gap width, gap) to add to the base model for successive model comparisons. These two variables were only moderately correlated (r = 0.464); because the correlation is positive the effect on predictions would be to diminish the effect size of each, so inclusion of both could be considered to give conservative estimates of effect size. The coefficient for an effect of wet season was positive as predicted, but the effect was non-significant ($P_{sea} = 0.828$). Effect sizes of traffic volume and road gap are depicted in Figure 5.4A.

Carrying the average traffic and gap width fixed effects and the random site effect over as the base model for snake-level effects ($atr + gap + [site]$, Table 5.1B), SVL was the only significant intrinsic predictor of road crossing rates ($P_{svl} = 0.015$) with a positive effect (i.e., larger snakes being more likely to cross roads) as predicted. After censoring 26 movement records of 9 snakes too small to be sexed, there was no significant difference between sexes in crossing rates ($P_{sex} = 0.174$) nor was there a significant interaction between sex and SVL. In classifying snakes by reproductive class (cls) there was a significant difference in crossing rates among juvenile, intermediate and adult groups, but these differences were consistent with the effect of increased SVL. In a *post hoc* model including movements of only adult snakes there was no difference between crossing rates of adult males and adult females. Therefore, SVL more parsimoniously explains crossing rates and was selected as a base term for successive model comparisons. The coefficient for an effect of body condition

Table 5.1: List of fixed effects considered for model inclusion, with observed values for continuous variables and sample sizes for categorical variables. "Effect" is the hypothesized/observed influence of the factor (i.e., sign of the regression coefficient; "NS" = not significant). P_{sole} is the P-value for the regression coefficient when it was added alone to the level-respective base model, followed by the ΔAICc value (differences > 2 AICc units are considered to have adequate support for inclusion in models). P_{final} is the P-value for the term in the final model (bottom). "*" indicates that the effect of sex was assessed in a separate model eschewing observations of snakes too small to determine the sex, and was therefore not included in the same AIC model selection process as the other snake-level effects.

Code	Factor	Units	Range	Mean ± SD	Effect	P_{sole}	ΔAICc	P_{final}
A) Site-level effects (n = 50; base model = [*site*])								
gap	Width between forest edges	m	3.5–27.7	11.5±5.6	–/–	**0.011**	**-4.9**	**0.015**
min	Minimum width of gap	m	2.6–26.4	9.6±5.5	–/–	**0.014**	**-4.5**	–
srf	Width of paved surface	m	0–11.6	6.46±1.7	–/–	**0.003**	**-6.4**	–
pav	Road surface (paved/unpaved)		n = 30 paved, 20 unpaved		**p–/p–**	**0.012**	**-3.9**	–
atr	Average traffic (vehicles/night)	v/n	0–3972	259±786	–/–	**<0.001**	**-14.3**	**0.007**
sea	Season (wet/dry)		n = 26 wet, 24 dry		w+/w+(NS)	0.828	+1.9	–
B) Snake-level effects (n = 691; base model = *gap* + *atr* + [*site*])								
svl	Snout-vent length	mm	381–1525	943±149	+/+	**0.015**	**-4.0**	**0.021**
*sex**	Sex (female/male)		n = 367 females, 315 males		m+/m–(NS)	0.174	–	–
rpr	Reproductive class (juv/int/adf/adm)	n = 249 juv, 269 int, 78 adf, 95 adm			?/?	0.012–0.270	-1.2	–
ci	Body condition index		Standard normal (0±1)		+/+(NS)	0.548	+1.6	–
C) Trial-level contextual effects (n = 2785; base model = *svl* + *gap* + *atr* + [*site*])								
dst	Snake's distance from edge	m	0–165	19±20	–/–	**0.003**	**-9.7**	**<0.001**
trp	Mouse-baited traps (y/n)		n = 632 yes, 2153 no		–/–	**0.036**	**-3.0**	**0.004**
ntr	Nightly traffic count (vehicles/night)	v/n	0–4237	235±740	–/–(NS)	0.496	+1.6	–
D) Trial-level environmental effects (n = 2785; base model = *dst* + *trp* + *svl* + *gap* + *atr* + [*site*])								
mn	Moonlight index	index	0–6.5	2.7±2.1	–/–(NS)	0.546	+1.6	–
cmn	Cloud-adjusted moonlight index	index	0–5.9	2.1±1.8	–/–(NS)	0.378	+1.2	–
pr	Precipitation (NWS)	mm/hr	0–7	0.24±0.63	+/+(NS)	0.501	+1.6	–
prg	Precipitation (plastic rain gauge)	mm	0–88	6.7±13.9	+/+(NS)	0.324	+1.0	–
hum	Humidity	%	67–96	0.84±0.06	+/+	**0.003**	**-6.4**	**0.003**
tmp	Temperature	∘C	23.8–28.8	2.69±1.1	+/+(NS)	0.959	+2.0	–
wnd	Wind speed	m/s	0.1–8.9	3.15±1.7	–/+(NS)	0.772	+1.9	–
gst	Wind gust	m/s	0–11.96	0.73±1.68	–/+(NS)	0.713	+1.9	–

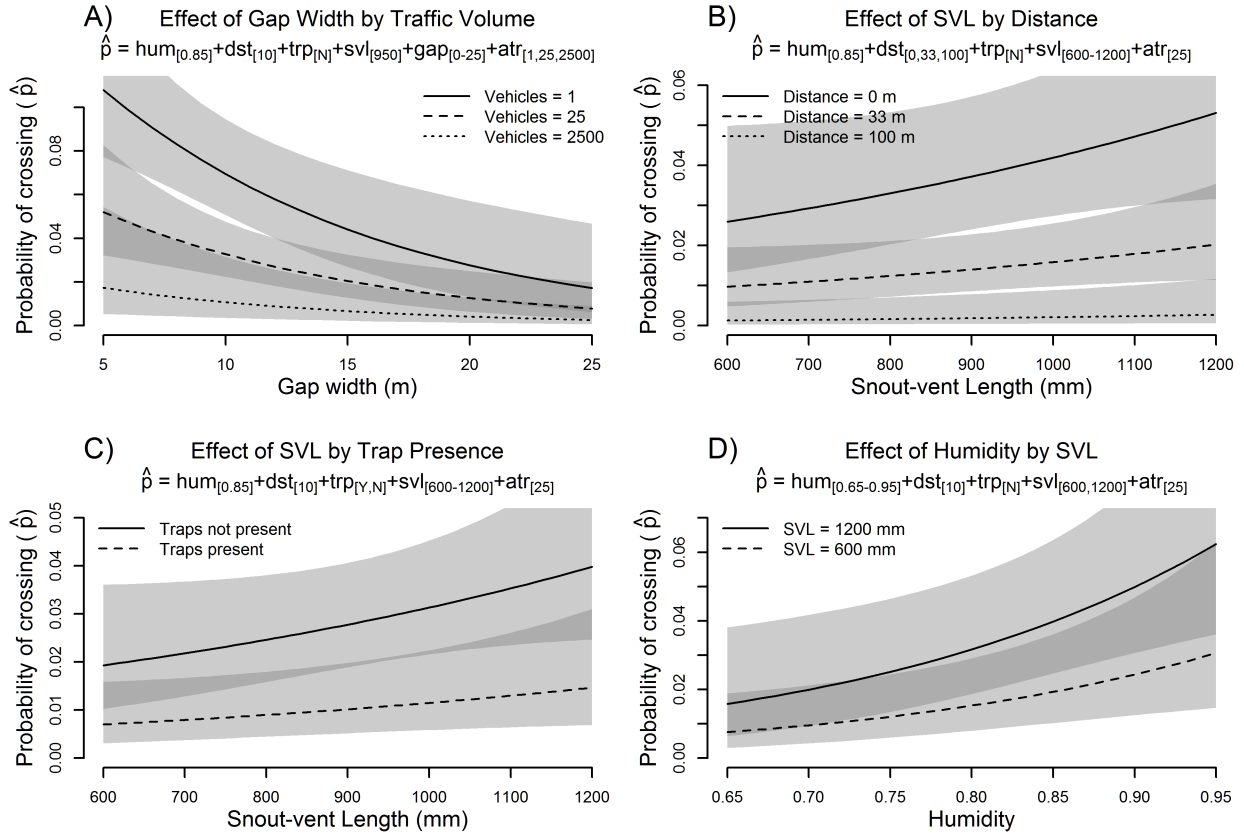**Final model:** *hum* + *dst* + *trp* + *svl* + *gap* + *atr* + [*site*]

Figure 5.4: Effect sizes of road, individual, contextual and environmental factors influencing road crossing rates of radiotelemetered Brown Treesnakes on Guam. Values associated with model terms ("[*value*]") are the values at which response curves were predicted. Gray polygons represent the 95% confidence intervals for the estimates (±1.96*SE).

was positive as predicted but the effect was not significant ($P_{ci} = 0.548$). The effect of SVL on road crossing rates is represented in Figures 5.4B and C.

Among contextual factors recorded at the trial level (Table 5.1C), distance and trap effects were highly significant. Snakes further from the forest edge at the start of the trial were much less likely to cross the road, as predicted ($P_{dst} = 0.003$; Figure 5.4B). On nights where mouse-baited snake traps were present, snakes were less likely to cross the road ($P_{trp} = 0.036$; Figure 5.4C). Nightly variation in traffic volume was negatively associated with crossing rates as predicted, but the effect was not significant ($P_{ntr} = 0.496$); variation among nights adds little beyond the highly significant effect of average traffic volume at a site. The terms *dst* and *trp* were carried over as part of the base model for assessing environmental

effects.

Moonlight, as represented by our *ad hoc* index, had a negative effect as predicted but was not significant ($P_{mn} = 0.546$). The cloud-adjusted index performed slightly better but was also non-significant ($P_{cmn} = 0.378$). Rainfall as measured by an on-site plastic gauge provided a slightly better fit than data from the NWS weather station, and effect estimates were positive as predicted, but both effects were non-significant ($P_{prg,pr} = 0.324, 0.501$). Humidity was the only environmental variable that was significant when included as the only additional term to the base model. When all possible combinations of terms were considered, combinations of humidity, temperature and wind speed were highly significant; however, these terms are highly negatively correlated and because humidity was the only significant term when included alone ($P_{hum} = 0.003$; Figure 4D) it was the only environmental variable we chose to add to our final model:

$$logit(p_{ijk}) = \beta_{int} + \beta_{hum_{ijk}} + \beta_{dst_{ijk}} + \beta_{trp_{ijk}} + \beta_{svl_{jk}} + \beta_{gap_k} + \beta_{atr_k} + \gamma_k$$

$$\gamma_k \sim N(0, \sigma_\gamma^2) \tag{1}$$

where the estimated probability of crossing (p) on trial i by snake j at site k is the product of the covariate values and the coefficient estimates (βs), offset by the [*site*] random effect for site k (γ_k) which is assumed to be a normal random variable with a mean of zero and a variance estimate of σ_γ^2.

As estimated by this model, all β effects were significant at $\alpha = 0.05$, with negative effects of traffic volume ($P_{atr} = 0.007$) and gap width ($P_{gap} = 0.015$), positive effect of snake size ($P_{svl} = 0.021$), negative distance and trap contextual effects ($P_{dst,trp} < 0.001, 0.004$) and a positive humidity effect ($P_{hum} = 0.003$). The variance estimate for the [*site*] random effect (σ_γ^2) was 0.333. This model constituted an improvement of fit of 41.36 AICc units over the base model of random site effect only. By this model, a relatively large snake (1200 mm)

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under very high crossing-probability conditions – directly on the forest edge at the beginning of the night on a road with no traffic, a 5-meter gap between forest edges, and 90% humidity – would be 27% likely to cross that road, with that probability dropping to 0.51% for an average (950 mm) snake 25m from the edge of a road with an average of 250 vehicles passing per night, a gap of 15 m, and a humidity of 80%. Under even lower-probability conditions (600mm snake, 50 m from road edge, 1000 vehicles per night, 25 m gap, 70% humidity) the probability of crossing would be vanishingly small (0.0167%).

I reported beta estimates from a simplified model using untransformed data in Table 5.2 in order to allow the reader to make estimations of crossing probabilities based on new data in the native scale. Mixed effects models would not converge when using untransformed data, so I report the coefficient and standard error values from a fixed effects model. Untransformed traffic values were not significant in the fixed effects model so I dropped the term from this simplified model and relied on gap width as an easily-measured proxy for road magnitude. The presence of traps (trp) was a categorical variable, with the natural trapless state being the baseline ("0") condition, so this term can be dropped ($0 * \beta_{trp} = 0$) when traps are not present at road edges. The simplified model is:

$$logit(p_{ijk}) = \beta_{int} + \beta_{hum_i} + \beta_{dst_i} + \beta_{trp_i} + \beta_{svl_j} + \beta_{gap_k} \tag{2}$$

and the equation for estimation of the probability of road crossing ($\hat{p}$), then, is:

$$p = \frac{1}{1 + e^{-(-10.01 + (7.94 * hum) + (-0.0323 * dst) + (-0.958 * [0,1]) + (0.00203 * svl) + (-0.126 * gap))}} \tag{3}$$

Using this simplified model, holding road, contextual and environmental conditions constant (gap, tr, hum), randomizing distance from the road (dst), and using actual snake sizes (svl) from sites differing in size distributions (Figure 5.5A), simulations demonstrated the potential for considerable differences among sites in absolute numbers of road crossings events resulting from differences in snake size among sites (Figure 5.5B).

Table 5.2: Fixed-effects betas (coefficient estimates) from the simplified model based on untransformed (native scale) predictor variables. New data for predictions should not exceed the range of values on which the model was based ("Range").

β	Factor	Range	Estimate	Std. Error	P-value
β_{int}	Model intercept (average)	NA	-10.01005	1.779558	$<< 0.0001$
β_{hum}	Humidity	0.67–0.96	+7.94182	1.993966	<0.0001
β_{dst}	Distance from edge (m)	0–165	-0.03225	0.008618	0.0002
β_{tr}	Traps present (0 = no, 1 = yes)	0,1	-0.95761	0.331804	0.0039
β_{svl}	Snake snout-vent length (mm)	381–1,525	+0.00203	0.000733	0.0055
β_{gap}	Gap width (m)	3.5–27	-0.12591	0.027693	$<<0.0001$

Discussion

Our results indicate strong influence of a multitude of extrinsic factors on the nightly probability that a Brown Treesnake on Guam will cross a road. All metrics of increasing road magnitude had significant negative effects, reducing crossing probabilities. Disentangling which specific factors add which components of variation among these highly correlated metrics is non-trivial. While this may be desirable from a behavioral standpoint, the point may be relatively meaningless in practice when predicting which roads are likely to be crossed more often, as roads tend to be wider and more likely to be paved as traffic volume increases. Traffic levels are readily measurable with pneumatic roadside units given modest effort and expense. However, we suggest that gap width may be a more easily-measured, though less precise, predictor of crossing rates. That gap width remains significant when accounting for traffic levels and random site effects suggests a behavioral aversion to terrestrial movement and/or exposure upon leaving forest cover. For an individual snake on a given night, extrinsic factors such as the location of the snake with respect to the road and the presence of local prey cues had strong effects on probability of crossing a road. While we had a case for predicting a strong effect of moonlight, precipitation, and season on road crossing rates, the only environmental effect with which we were able to demonstrate a significant relationship was humidity, with fewer crossings on less humid nights.

The presence of snake traps baited with live mice was initially considered a regrettable
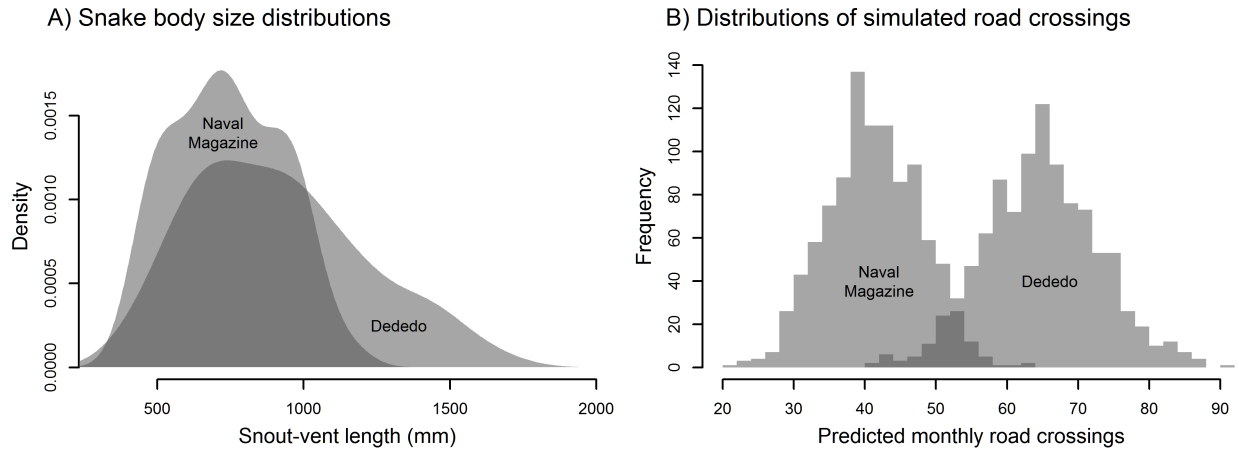
Figure 5.5: Size distributions of samples of 100 snakes each from two sites on Guam (A), and simulated differences in predicted road crossing events per month (B) resulting from differences in snake size distributions based on the simplified road crossing prediction model. Simulations were run over 1000 iterations, with randomized distances from the road (0 to 100 m), standardized humidity (85%) and gap width (10 m), and absence of mouse-baited snake traps.

nuisance effect of observer interference with the system being studied, but in retrospect offers potentially valuable insight into the behavioral decisions made by snakes. It appears that the presence of strong prey cues – as resulting from the presence of bait mice in traps – had an effect of reducing or localizing the movement of snakes, at least with respect to probability of road crossings. Forest habitats on Guam are depauperate of the bird and mammal prey preferred by larger Brown Treesnakes (Savidge, 1988; Chapter Three), and we presumed that both sides of a surveyed road segment were at an equilibrium with respect to prey densities. While these data cannot demonstrate an increased rate of road crossing by snakes following stronger prey cues from the opposite side of the road, they do demonstrate a reduced rate of road crossing when local prey cues are artificially high. We encourage future research on the effect of prey density differentials on road crossing rates of Brown Treesnakes and other snake species.

Snake size was the only intrinsic factor appearing to influence road crossing probabilities. This positive effect of SVL was consistent with observations of increased terrestrial loco-motion by larger Brown Treesnakes (Rodda and Reed, 2007) and greater movement rates

and road use by larger snakes of other species (Brito and Álvares, 2004; Andrews and Gibbons, 2008; Hyslop et al., 2014). If road crossing avoidance results from a reluctance of snakes to depart forest cover as a form of antipredator behavior, as suggested by the gap width effect, it may be logical that such avoidance is greater in smaller snakes that may be more vulnerable to a wider variety of predators (Mushinsky and Miller, 1993; Bittner, 2003). This antipredator behavior may decline with growth of the snake (e.g., Roth and Johnson, 2004). Alternatively and non-exclusively, smaller Brown Treesnakes prey almost exclusively on small lizards (Savidge, 1988; Lardner et al., 2009; Chapter Three) – which are ubiquitous and hyperabundant (Campbell, III et al., 2012) – while larger snakes tend to take bird and mammal prey which are rare and heterogeneously distributed in Guam (Savidge, 1988; Chapter Three). This may potentially lead to a greater drive for inter-habitat movements by larger snakes seeking size-appropriate prey. During my observation of 2945 nightly movements of 728 snakes within close proximity to a road, I observed only one snake mortality, a 1,271-mm male found dead near the forest edge on the shoulder of a high-traffic road. This snake was at the 97$^{\text{th}}$ percentile of snake sizes monitored during this study. I was unable to determine if the snake had been killed by vehicle collision or by other causes. If so, this might support the trend of larger snakes more frequently crossing and being killed on roads (Andrews and Gibbons, 2008; Shepard et al., 2008a).

Very large snakes that are more likely to exhibit reproductive behavior were underrepresented in this study. While we were unable to demonstrate differences in road crossing rates by snakes considered to be fully mature based on size classes, true reproductive status cannot be verified without necropsy and no obviously gravid snakes were encountered during our sampling. While the potential exists for road crossing behavior to vary among actively reproductive snakes, we were not able to demonstrate such differences. Large reproductive snakes are relatively rare on Guam, so crossings by this small proportion of individuals are not likely to have a large impact on the overall number of road crossings.

My method of monitoring the nightly relocation distances of snakes makes re-crossings

of a road within a single night undetectable. However, the rarity of crossing events and the very low rates of visual detection of snakes on roads or within road gaps suggest that such "double-crossing" events must be rare. Over the course of more than 2,300 hours of recent Brown Treesnake surveys along habitat edges at 18 sites during optimal snake activity hours (Chapters One through Three), we made no observations of snakes on roads. As such, I am confident that my results accurately characterize the factors affecting the majority of road crossings by snakes on Guam.

Per capita, road crossings as modeled here are relatively rare events, consistent with published assessments of road crossing avoidance by Brown Treesnakes (Siers et al., 2014) and other snakes (e.g., Shine et al., 2004; Andrews and Gibbons, 2005; Shepard et al., 2008b; Miller et al., 2012; Robson and Blouin-Demers, 2013). Actual numbers of snakes crossing a road during a given timeframe will depend on additional factors such as snake density and distribution with respect to the road. My results demonstrate that snakes closer to road edges are more likely to cross the road; however, these data cannot demonstrate what proportion of snakes utilizing the landscape were within close proximity to the road. Affinity for edge habitat has been documented in multiple snake species (e.g., Blouin-Demers and Weatherhead, 2001) and Brown Treesnakes appear to have no aversion to forest habitat at road edges, as this is where the majority of visual observations of foraging snakes occur. If snake density is greater in edge habitat, this will increase actual crossings compared to a uniform distribution of snake densities across the landscape. Given the positive effect of snake size on road crossing probabilities, variation in the size distributions of snakes at a given site may modulate the actual numbers of road crossing events that occur within a given time period (Figure 5.5).

Brown Treesnakes can be effectively eradicated from discrete habitat units if immigration can be controlled (Rodda et al., 1999a, 2002), but their high mobility makes this difficult to achieve (Savarie et al., 2001). Future plans to suppress Brown Treesnake populations involve aerial broadcast of toxicant-laden baits at the landscape scale (Savarie et al., 2001; Clark

and Savarie, 2012). Out of logistic necessity, not all areas can be treated at the same time, and it is likely that roads will form *de facto* boundaries for management units. Information on predictable "leak-back" rates from untreated areas will be important for modeling the prospects for success of such efforts, and this understanding of road, context, and snake characteristics on the partial barrier effects of roads may make such estimates feasible. An understanding of the effect of roads on landscape-scale movements of invasive snakes may also be useful in predicting the rates of spread of novel invasions. Variation in crossing probabilities by snake size introduces a "filter effect," where cross-road migrants will not be a random subset of snakes but rather a size-biased sample of larger snakes. This will have demographic consequences when considering novel invasions or re-invasions across barrier roads.

Movement and road crossing rates of Brown Treesnakes on Guam appear to have declined in the ~60 years since invasion. Qualitative descriptions by Santana-Bendix (1994) and Tobin et al. (1999) differ in that Santana-Bendix described the movements of snakes tracked between 1988 and 1990 to be without defined activity areas, while Tobin described snakes tracked in 1996 as having greater site fidelity. McCoid and Hensley (2000) recorded 184 roadkilled Brown Treesnakes during 20.4 km of daily driving over two years, a rate that appears extraordinarily high compared to contemporary but unquantified observations of roadkilled snakes (Siers, pers. obs.). Rodda et al. (2008) hypothesized that ecological conditions on Guam prior to the irruption of Brown Treesnakes favored "high-moving" snakes that dispersed into prey-rich unoccupied habitats, with a reversal in selection for low movement and energy conservation subsequent to the collapse of populations of larger prey species. Fahrig (2007) suggests that wildlife populations that have inhabited landscapes with high habitat cover have evolved low boundary responses and high movement probabilities, making them highly susceptible to increased movement mortality resulting from habitat loss and reduced matrix quality. It is not hard to conceive that Brown Treesnakes originating in low-development areas may have historically crossed roads indiscriminately, but individ-

uals more prone to extensive movements and gap-crossing behavior may have been selected against by on-road mortality over the 60 years since invasion, resulting in a population that crosses roads less frequently.

By focusing our efforts on a large number of animals at a large number of sites for the relatively short periods during which they were in close proximity to roads, we were able to elucidate a hierarchy of factors affecting road crossing rates by invasive Brown Treesnakes on Guam. These data are the first to provide managers with a reasonable baseline assessment of the barrier effect of roads on landscape-level movements of these costly invasive predators, and one of a very small number of studies that simultaneously assesses intrinsic and extrinsic factors affecting individual probabilities of road crossing by wildlife. Similar methodologies may be useful for those studying the movement of other organisms with respect to roads or other landscape features. With the global proliferation of roads due to by development and resource exploitation, it will become increasingly important that we move from qualitative assessments of the effects of roads to a greater understanding of the multivariate, quantitative and cumulative effects of roads on wildlife and how those effects vary among and within species.

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